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Alterations in brain-cerebrospinal fluid (CSF) cations have been shown to affect many physiological systems (e.g., temperature regulation, circulation, respiration, etc.). Moreover, numerous investigators have noted changes in brain-CSF cations during stress (e.g., hypoxia, hypothermia, etc.) and these changes seem to occur concomitantly with the activation of the hypothalamo-hypophyseal-adrenocortical (HHA) system. Based on these considerations, the present study was designed to investigate whether alterations, within

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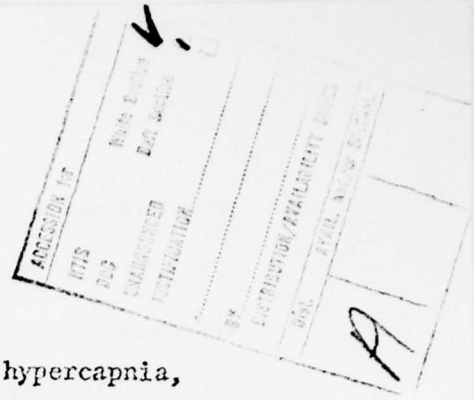
physiological ranges, in the concentrations of various CSF cations (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+}) could affect the basal activity of the HHA system directly or through their actions on neurotransmitters which are known to modulate the activity of the hypophysiotropic area.

Adult male and female cats were prepared with left lateral cerebroventricular and right atrial cannulae. After establishing that the HHA system of this animal model was in a basal state and responsive to exogenous stimuli, the cerebroventricles of conscious unrestrained cats were perfused with normal mock CSF or CSF containing high or low concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} or Li^+. Blood samples were taken at various times during CSF perfusion and analyzed for cortisol fluorometrically. These studies demonstrated that although elevated CSF $[K^+]$, $[Ca^{2+}]$ or $[Mg^{2+}]$ did not alter plasma cortisol levels, reducing these CSF cations activated the HHA system, possibly by hyperpolarizing an inhibitory neural path(s) to CRF neurons. Altering CSF $[Na^+]$ and $[Li^+]$ did not affect HHA activity.

The excitatory effect of the low concentrations of cations prompted further investigations as to whether the actions of these cations were mediated via adrenergic, cholinergic and/or gabanergic neural systems, however, before pursuing these studies the influence of these neural systems on basal HHA activity was ascertained. Cats were perfused with normal CSF containing various receptor antagonists. The alpha and beta adrenergic receptor blockers (phenotolamine and propranolol) administered alone or together elevated cortisol levels, whereas neither nicotonic and muscarinic cholinergic (mecamylamine and atropine) nor gabanergic (picrotoxin) blockers affected basal HHA activity. When all five blockers were perfused simultaneously only the excitatory action of the adrenergic blockers was noted. The consideration of whether the action of reduced CSF cations was mediated via the adrenergic system prompted the perfusion of cats with CSF containing norepinephrine and a lack of Ca^{2+}. The excitatory action of reduced $[Ca^{2+}]$ was inhibited by norepinephrine, suggesting that the lack of this cation inhibited the release of the adrenergic neurotransmitter.

A final consideration was to ascertain whether the excitatory effects of reduced CSF cations and receptor antagonists acted via the feedback site(s). The cerebroventricles of cats were perfused with dexamethasone together with reduced CSF cations or receptor blockers. The stimulatory actions of the low concentrations of cations and the adrenergic receptor blockers were inhibited by dexamethasone.

These data suggest that in the conscious unrestrained cat basal activity of the HHA system is: 1) influenced by the concentrations of CSF cations; 2) maintained primarily by the inhibitory action of the adrenergic system and the latter is dependent upon normal extracellular $[K^+]$, $[Ca^{2+}]$ and $[Mg^{2+}]$; and 3) not maintained by the cholinergic or gabanergic system nor is the effect of the adrenergic system mediated via the cholinergic and/or gabanergic systems. In addition, the excitatory action of lowered CSF cations and the inhibitory effect of the adrenergic system on basal HHA activity seem to act through the feedback site(s). Thus, it is postulated that those stressors which lower CSF cations may at least partially activate the HHA system by relaxing the tonic inhibitory action of the adrenergic system.



I. LITERATURE REVIEW

Many environmental stressors such as hypoxia, hypercapnia, hypothermia, etc. have been shown to enhance adrenocortical activity; however, the mechanism(s) whereby these stressors affect the hypothalamo-hypophyseal-adrenocortical (HHA) system is not readily known. It has been proposed that the adrenocortical stimulus may be mediated initially through peripheral respiratory chemoreceptors for acute hypoxia (76, 78, 87) and the hypothalamo-hypophyseal complex for acute hypercapnia (77, 79). These stressors, as well as others, have also been shown to affect acid-base balance and ultimately the $[H^+]$ of cerebrospinal fluid (CSF). The proximity of the hypothalamo-hypophyseal complex to the ventricular system and CSF led Malasanos and Marotta (83, 84) to investigate whether changes in CSF $[H^+]$ would affect HHA activity as well as respiratory parameters. They showed that in nonstressed anesthetized dogs perfusion of the cerebroventricles with elevated CSF $[H^+]$ stimulated, while decreased CSF $[H^+]$ inhibited, adrenocortical secretory rates. Furthermore, the perfusion of basic CSF attenuated the adrenocortical response to hypoxia, whereas acidic CSF augmented this response.

In addition to changing CSF $[H^+]$, various stressors are known to alter other brain-CSF cations which in turn could affect central neural (e.g., hypothalamic) activity. Dogs subjected to lowered ambient oxygen (O_2) concentrations exhibit not only respiratory alkalosis but also decreases in CSF $[K^+]$. The inhalation of high

concentrations of carbon dioxide (CO_2) leads to an elevation in CSF [K^+] (14). Furthermore, radiolabelled $^{22}Na^+$ increases and $^{45}Ca^{2+}$ decreases in CSF when hyperpyrexia is produced by injecting a pyrogen, Salmonella typhosa, into the lateral ventricle of conscious cats (104).

Alterations in various CSF cations induced either by intraventricular injections of cations or environmental stressors are also capable of affecting body temperature, respiratory rate, heart rate, arterial pressure, thirst and hunger. The cerebroventricular or push-pull (posterior hypothalamus) perfusion of unanesthetized cats and monkeys with CSF containing markedly elevated [Na^+] has been shown to increase body temperature, whereas perfusion with high [Ca^{2+}] decreased body temperature (36, 98, 100, 101, 102). Excess K^+ or Mg^{2+} had essentially no effect on body temperature. The altered temperatures persisted until the [Na^+] and [Ca^{2+}] returned to normal levels. Thus, a new "set-point" was established and the animals were capable of maintaining this newly acquired temperature when challenged by a hot or cold stressor (102). These investigators postulated that body temperature is maintained at 37°C by "inborn enzymatic" functions of posterior hypothalamic neurons that regulate the release of a neurotransmitter (e.g., acetylcholine) depending upon the ratio of Na^+ to Ca^{2+} in the extracellular fluid (98); however, the high concentration of Na^+ and Ca^{2+} used in these studies may have nonspecifically stimulated and inhibited, respectively, the heat gain center (41). Others have proposed that it is not necessary to postulate a "set-point", since osmoreceptors in the preoptic area of the hypothalamus (48) may detect changes in the Na^+/Ca^{2+} or osmolarity and transduce these into signals

to the temperature regulating centers (124).

Changing the concentrations of K^+, Ca^{2+}, or Mg^{2+} in various limbic sites can influence respiration, heart rate and arterial pressure (94, 106, 117). Within three minutes after the microinjection of 200-800 µg K^+ into the cat's infundibulum respiratory rates increased (16), while a 25% KCl solution injected into either the hippocampus or cerebroventricles of rats (4) and dogs (28), respectively, increased heart rates. On the other hand, hypoventilation and/or bradycardia occurred when Ca^{2+} or Mg^{2+} was elevated in the hypothalami of the cat (16), rat (16) and rabbit (24). The effects of elevated $[K^+]$, $[Ca^{2+}]$ and $[Mg^{2+}]$ on respiration and heart rate may be attributed to excitation and/or depression of neural centers located within the brain stem. The perfusion of CSF containing a lack of Ca^{2+} or an excess of K^+ through the cerebroventricles of anesthetized dogs caused marked elevations in arterial pressure, while excesses of Ca^{2+} and Mg^{2+} caused hypotension (81). The pressor activities of low Ca^{2+} and high K^+ were related to their stimulatory effect on the vasomotor center which in turn stimulated the release of catecholamines from the adrenal medulla (81). The decrease in arterial pressure caused by an excess of Ca^{2+} or Mg^{2+} was considered a depressive action of these cations on this center (81).

Changes in CSF $[Na^+]$, $[K^+]$ and $[Ca^{2+}]$ also have been shown to alter hunger and thirst. Microinjection or push-pull perfusion of excess Na^+, K^+ or Ca^{2+} in the lateral hypothalamus of satiated or hungry animals increased the quantities of food ingested (33, 99, 103). Furthermore, hypertonic saline (1.5%) or a 25% KCl solution injected

into various hypothalamic sites (e.g., anterior, lateral, and dorsal) of the rat (33), rabbit (112) or goat (3) motivated the animals to drink within minutes after the injection. The increased drinking occurs when osmoreceptors in the hypothalamus are stimulated by the increased tonicity of the extracellular fluid (3).

Although little information is available on the actions of CSF cations on HHA activity, the alterations in brain-CSF Na^+, K^+, Ca^{2+} and Mg^{2+} caused by several environmental stressors, as well as the effects that experimentally induced changes in brain-CSF cations have on various physiological and behavioral activities, make it tempting to postulate that these CSF cations may also affect the activity of the HHA system. Although specific mechanism(s) describing the regulation of physiological activities by brain-CSF cations are not readily understood, the probable actions of these cations on the HHA system may be 1) directly on primary sites (i.e., CRF neurons and/or adenohypophysis); 2) indirectly on secondary sites (i.e., neural systems or paths) which may exhibit adrenergic, cholinergic and/or gabanergic activity; and 3) a common neural pathway (feedback site) that could be affected by these neurotransmitters.

The effects of monovalent and divalent cations directly on neural tissue have been known for some time. The presence of Na^+ is essential for the rate of rise and amplitude of the action potential (59, 60). These two components of the action potential are proportional to the log of the ratio of extra- to intracellular Na^+ (59). In addition, Na^+ has been shown to 1) affect the release of neurotransmitters from the presynaptic terminals; 2) be necessary for the generation of excitatory

postsynaptic potentials; and 3) be involved in the re-uptake of the neurotransmitter by nerve endings (8, 11, 45, 59). Although Li^+ may be substituted for Na^+ in maintaining neural function (60), this ion decreases the release of norepinephrine and serotonin from the rat's brain (21, 55, 58), increases the uptake of monoamines and modulates tryptophan hydroxylase activity (65, 68, 85). A combination of these actions of Li^+ may lead to the normalization of monoamine content at synapses with the subsequent amelioration of the manic or depressive state in human patients (21, 55, 58, 65, 85). Furthermore, activation of neurons can occur when extracellular $[K^+]$ is markedly increased (45, 53, 75, 96). These effects of K^+ include depolarization of the axonal membrane for the propagation of an action potential and/or depolarization of the presynaptic terminal resulting in neurotransmitter release and the subsequent increase in synaptic activity (45, 53, 75, 96, 97).

Neurons and other secretory tissues may be excited by Na^+ and K^+; however, the presence of normal extracellular $[Ca^{2+}]$ is essential for the coupling of stimulus and secretion of neurotransmitters and hormones (6, 8, 32, 114, 115, 127), while Mg^{2+} is involved in the active uptake of neurotransmitters into the storage vesicles (113). An additional function of normal $[Ca^{2+}]$ is the generation of the spike in an action potential (32, 60). When the extracellular $[Ca^{2+}]$ of neurons is reduced, spontaneous discharges occur in the form of the action potential (37, 94) which is attributed to an increase in conductance of monovalent ions (37, 107). On the other hand, low $[Mg^{2+}]$ does not appear to affect neural activity (60). The ionotophoretic application

of high [Ca^{2+}] or [Mg^{2+}] to neurons decreases neural activity (125). This suppressive effect appears to be localized in the postsynaptic activity of neurons (125). In contrast, increased [Mg^{2+}] and [Ca^{2+}] at the neuromuscular junction are antagonistic to each other. The decreasing activity caused by Mg^{2+} can be restored by increasing Ca^{2+} (12, 60). The antagonistic action between Mg^{2+} and Ca^{2+} is a presynaptic competitive effect which reduces the release of acetylcholine (60).

The numerous effects of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} on the activity of neurons, as well as other cells, suggest the posssibility that these cations could act directly on various components or levels of the HHA system. Although the presence of extracellular Ca^{2+}, but not K^{+}, is required for the secretion of glucocorticoids from adrenocortical cells (56), this component of the HHA system can be excluded from being affected when CSF cations are changed directly, since CSF and the systemic circulation are essentially separated and maintained by different mechanisms (23). Even though CSF cations could enter the systemic circulation during the perfusion of the cerebroventricles, the marked dilution of these ions would render them ineffective on adrenocortical cells.

The remaining two components of the HHA system, the CRF neurons and the chromophobes of the adenohypophysis, could be affected directly by CSF cations if a connection is provided between the cerebroventricular system and the hypothalamo-hypophyseal complex. An association between the third ventricle and the CRF neurons has been demonstrated. CSF cations may diffuse directly into the parenchyma

and/or be taken up actively by specialized ependymal cells, the tanycytes. These cells send processes juxtaposed to the hypothalamic neurons and may secrete into the hypothalamo-hypophyseal portal vessels (47, 109). Thus, the latter may act as a possible transport channel between the cerebroventricles and the adenohypophysis. Recently it has been shown that when the luteinizing releasing factor (LRF) is injected into the third ventricle of the rat, the luteinizing hormone (LH) is released (5, 110). Furthermore, following the administration of NaI^{131}, LH-I^{131}, ^{3}H-corticosterone or ACTH-I^{125} into the cerebroventricles, these substances appeared in the median eminence, infundibular stalk and single portal vessels (62, 116). Thus, it is conceivable that CSF cations could be transported to the adenohypophyseal extracellular space and affect adenohypophyseal activity. Further credence is given to this hypothesis when one considers that the corticotropin releasing factor (CRF) and adrenocorticotropin (ACTH) are released from in vitro preparations of rat's hypothalamic (6) and adenohypophyseal slices (69, 70, 90), respectively, when incubated in the presence of 30-55 mM K^{+} and 0.75 mM Ca^{2+}.

In addition to the possible direct effects of CSF cations on CRF neurons and/or the anterior pituitary, the monoamines (e.g., norepinephrine, dopamine and serotonin) which have been located in the hypothalamus (19, 38, 40, 52, 77, 111, 119), could serve as indirect mediators of CSF cations by the latter altering the synthesis, storage, release and/or uptake of these neurotransmitters. The monoamine, serotonin, when injected into the anterior hypothalamus of cats and monkeys increases body temperature, whereas lowering the body

temperature of these animals causes hypothalamic release of serotonin (105). These studies suggest that this monoamine affects the heat gain mechanism. Evidence of a role for serotonin in regulating HHA activity is conflicting. When serotonin is lowered in the rat's brain by a diet deficient in tryptophan or the intraperitoneal administration of para chlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase, neither the basal nor the acute stress levels of plasma corticosterone are affected (29, 30); however, others have shown that 1) pCPA can act as a non-specific stressor in rats (89); 2) serotonin injected into the hypothalamus of hypothalamic deafferented guinea pigs is stimulatory (108); and 3) pCPA does not affect basal cortisol secretory rates of dogs, but does inhibit the adrenocortical response to hypoxia (88). On the other hand, when the fornix is transected or the animals are pretreated with pCPA, the serotonin levels in the hippocampus, which normally parallel plasma corticosterone levels and thus may be involved in circadian adrenocortical variations, decrease and a concomitant disruption of the circadian corticosterone rhythm occurs (27, 121). Thus, the evidence although incomplete indicates that the primary action of serotonin may be in the maintenance of circadian adrenocortical activity. Further determination of whether serotonin is involved in basal HHA activity cannot be fully ascertained until a blocker specific for serotonin receptors is found. The blockers (e.g., methysergide, cyproheptadine, etc.) currently available have many muscular and neural effects in addition to blocking serotonergic receptors (31).

Other monoamines that are known to influence various physiological

functions as well as possibly the CRF neurons include dopamine and norepinephrine. When norepinephrine is injected into the lateral hypothalamus of rats (46) and monkeys (106) drinking and/or feeding increases, whereas injection of this monoamine into the anterior hypothalamus of these animals decreases body temperature (105). The administration of bis-(1-methyl-4-homopiperazinyl-thiocarbonyl)-disulfide (FLA-63), an inhibitor of dopamine hydroxylase which elevates brain dopamine but depletes norepinephrine levels, elevates basal plasma corticosterone levels of rats (89, 120, 121). Furthermore, intravenous or cerebroventricular administration of norepinephrine into rats (10, 39, 86, 128) and dogs (42, 129) lowers plasma 11-hydroxycorticosteroid (11-OHCS) levels during basal or stress conditions, suggesting that the noradrenergic system exerts an inhibitory action on HHA activity. However, the inhibitory action of injected norepinephrine on plasma 11-OHCS levels does not indicate whether the endogenous monoamine is affecting alpha (α) and/or beta (β) adrenergic receptors during these two states of HHA activity. Only limited data are available concerning the type of adrenergic receptor involved in the inhibition of 11-OHCS release. When phentolamine (α-blocker) or propranolol (β-blocker) is injected intravenously or intraventricularly into nonstressed rats, corticosterone levels are markedly increased with the α-blocker but not with the β-blocker (121), while in laporatomized (121) or hypoxic rats (89) the inhibitory effect of norepinephrine appears to be mediated by the α-receptors in the former stress and both α- and β-receptors in the latter stress. Whether the inhibitory action of norepinephrine on HHA activity, which is partially mediated through

α- and/or β-receptors during basal and stressful conditions in the rat, is similar in other animal models (e.g., cat) remains to be ascertained.

The cholinergic neural system, which is involved in the regulation of numerous physiological functions, may also be considered in mediating the effects of altered CSF cations on HHA activity. These may include altering the release and/or uptake of acetylcholine (59, 107). Microimplantation of acetylcholine or the cholinomimetic carbacol into the anterior hypothalamus of monkeys increases body temperature, whereas when injected into the posterior hypothalamus it decreases body temperature. This suggests that the anterior hypothalamic cholinergic system is concerned with heat gain, whereas the posterior hypothalamus is involved with heat loss (105). When low doses of acetylcholine or carbacol are injected into the lateral hypothalamus of the monkey (106) or the rat (46) drinking is increased, while the administration of high doses of carbacol, which stimulates both the nicotinic(n) and muscarinic (m) receptors, into the basal hypothalamus of nonstressed conscious cats elevates plasma cortisol levels (74). When high doses of atropine which can block both n- and m-receptors (54) are injected into the same sites, the excitatory action of the agonist on the HHA system is blocked (74), indicating that either the n- and/or m-receptors exert a regulatory action on plasma cortisol levels; however, whether carbacol affected basal HHA activity was not determined since plasma cortisol levels were measured only in atropine plus carbacol treated animals. Furthermore, the implantation of atropine crystals in the anterior

hypothalamus of rats attenuates the adrenocortical response to the surgical stress of implantation (49), whereas the subcutaneous (71) or intravenous (72) injection of atropine into cats a few hours prior to the circadian rise in plasma cortisol levels blocks this elevation. Thus, the central cholinergic neural system may contribute to the regulation of HHA activity; however, whether n- and/or m-receptors affect this system during stressful, circadian and basal activities is not readily known.

The gabanergic neural system has been investigated as to its function in the central nervous system (CNS). The neurotransmitter, γ-aminobutyric acid (GABA), of this system inhibits neural activity presumably by increasing the conductance of the anion Cl^- (1, 93). This acid which has been located in cerebrocortical and subcortical structures (63, 93) has been considered an important regulatory factor in cortical pyramidal cell activity (93). Subcortically GABA content has been shown to decrease in the rat's lateral hypothalamus and increase in the ventromedial hypothalamus during hypoglycemia, while the reverse occurs during hyperglycemia (63). Thus, many investigators have postulated a role for GABA in regulating appetite. Furthermore, the granular cell layer of the hippocampus has been shown to contain GABA (126) which in turn reduces electrical discharges from adjacent pyramidal cells (17). Since stimulation of the hippocampus can decrease 11-OHCS levels (66, 91), the inhibitory effect may be via pyramidal neurons which in turn could be regulated by GABA neurons of the hippocampus. On the other hand, when GABA is injected into the median eminence of cats, plasma cortisol levels increase (73). This

suggests that a gabanergic neural system could be acting in the hypothalamus to suppress the action of inhibitory neurons on CRF neurons. In view of the limited information concerning the function of GABA in modulating adenocortical activity, further work is required in order to determine the role of this neural system in the regulation of basal HHA activity.

The neurotransmitters discussed above may act not only on neural paths to the CRF neurons but also on the CRF neurons themselves. Support for the latter was recently provided by Burden et al., (13), who studied the effects of various neurotransmitters on the in vitro release of CRF from rat's hypothalamic preparations. They found that acetylcholine and serotonin increased the release of CRF, whereas norepinephrine and GABA inhibited its release.

Proper function of the HHA system is dependent upon the appropriate action of the control (feedback) center(s) which quantitatively evaluates incoming excitatory and inhibitory signals, and then send a signal of an exactly determined intensity to CRF neurons (123). The control center or site that serves as a common input for various neurochemical and hormonal stimuli could be situated in the CNS and/or the adenohypophysis. Administration of cortisol or the synthetic glucocorticoid, dexamethasone, into the cerebroventricles or various subcortical sites (e.g., median eminence, etc.) depresses the stress responses and the circadian elevations in plasma 11-OHCS levels (20, 61, 130, 131). Thus, neurochemical or hormonal effects could be mediated via a common control center. Furthermore, the cytoplasmic and nuclear fractions of hypothalamic, hippocampal and adenohypophyseal

cells have a high affinity for glucocorticoids (44, 67, 92, 118), and the latter is known to depress hypothalamic and hippocampal neuronal activities (18, 35). This indicates that the pathways to the common site(s) can originate in various areas of the brain. Although injections of dopamine and serotonin into the third ventricle of rats cause the release of some trophic hormones, these monoamines have no effect when injected directly into the adenohypophysis (57). This suggests that neural mechanisms and their neurotransmitters, which affect hypothalamo-hypophyseal activity, would act through a common site(s) in the hypothalamus releasing hypothalamic factors and not directly on the adenohypophysis. Finally, Smelik (123) showed that when rats with hypothalamic dexamethasone implants are subjected to stress, the adrenocortical response is abolished; however, when a crude CRF preparation is injected into dexamathasone implanted rats, the adrenocortical response was normal (i.e., stimulated) for at least 24 hr before gradually declining (i.e., inhibition). These data suggest that initially glucocorticoid inhibition is at the hypothalamic level and gradually (after 24 hr) the sensitivity of the adenohypophysis decreases, presumably due to the absence of tonic hypothalamic influences on the anterior pituitary (67, 123).

Since changes in brain-CSF cations can affect various physiological activities, it is postulated that those mechanisms which regulate the ionic environment of the hypothalamus and are influenced by various stressors could affect HHA activity either directly and/or through neurotransmitters. Thus, the basic problem of this research project is to ascertain the role(s) of CSF cations in regulating HHA activity. In order to accomplish this, the following will be taken

under consideration once the conscious animal model is validated: 1) whether alterations, within physiological ranges, in the major CSF cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) can affect the basal activity of the RHA system; 2) whether Li^+, which is known to affect neurotransmitter activity, can partially replace Na^+ in CSF; 3) whether α-, β-, n-, m- and/or γ-receptors are involved in maintaining basal RHA activity; 4) whether the action(s) of CSF cations are mediated through adrenergic, cholinergic and/or gabanergic systems; and 5) whether the actions of CSF cations and neurotransmitters are directed to the feedback site(s).

II. MATERIALS AND METHODS

General Preparations

Seventy-four (254 trials-experiments) male and female adult cats, weighing 3.6 ± 0.1 kg, were maintained in separate cages in a constant temperature (25°C) room with a 12L:12D (lights on 0600 hr) lighting schedule and fed Wayne cat food (Allied Mills) and water ad libitum. The cats were anesthetized with Ketamine Hydrochloride (25 mg/kg; Parke, Davis and Co.) and a polyvinyl tubing (I.D. = .044"; O.D. = .065") was positioned in the superior vena cava-right atrium via the left external jugular vein. The cat's head was placed in a stereotaxic frame and an incision made along the skin of the skull. The muscles on the parietal bone were retracted and the skull cleaned with 10% hydrogen peroxide (J.T. Baker Chemical Co.).

The coordinates, 8 mm anterior to the earbars and 4 mm lateral to the midline (15), were located on the parietal bone and a hole (2 mm) was drilled through the bone. A modified electrode holder to which was attached a stainless steel block (12x12x12 mm) containing a bottom outlet with a 22G-1½" stainless steel cutting needle and two side outlets, one for monitoring pressure and the other for perfusing 0.9% saline, was lowered gradually into the brain. This procedure caused a gradual increase in pressure in the perfusion system until the lateral ventricle was reached. This was indicated by a sudden drop in pressure recorded on a Grass polygraph (Model 77B) via a Statham transducer (Model 23). The ventricular coordinate (13-15 mm) was

recorded and the cutting needle retracted. The latter was replaced with a 19G (1") needle cannula with a polyvinyl sleeve. While perfusing 0.9% saline through the needle to eliminate blockage of the cannula with brain tissue, the cannula was lowered into position. The external jugular cannula, which was brought under the skin of the neck and connected to another 19G needle, was attached to the top of the skull. This cannula as well as the lateral ventricular cannula were permanently affixed to the skull with acrylic cement (Fine Precision Dental Manufacturing Co.). The incision was closed with wound clips (14 mm) and the animal injected with one million units Bicillin C-R (Wyeth Laboratories, Inc.). Following the operation the animal was returned to its cage and checked daily for healing of wounds, patency of cannulae and general health. Following the post surgery experiment, all cats were allowed to recover for at least seven days prior to experimentation. When a cat was used for more than one experiment at least two days elapsed between experiments.

Validation of the Animal Model

In order to determine the time after surgery when cortisol levels return to non-stressed levels, blood samples (4-5 ml) were taken from 13 cats immediately following surgery and between 0800-1000 hr on days 1, 2, 3, 4 and 7 after surgery. These blood samples, as well as all subsequent blood samples, were stored in an iced bath until centrifuged at 2000 rpm for 12 min. Plasma was transferred to plastic vials and stored at -20°C until analyzed for cortisol fluorometrically (64).

In order to determine that the animal (20 trials on six cats) model's adrenal cortex was functioning, a large dose (20-25 units) of ACTH (Acthar; Armour Pharmaceutical Co. or Cortrosyn; Organon, Inc.) was injected intravenously (IV) into cats at least seven days post-operatively. Blood samples, which were analyzed for cortisol concentrations (64), were collected before and 5, 10 and 15-20 min after the IV infusion of ACTH. In addition, ACTH dose-response studies were performed on 22 cats so that the minimum dose of ACTH (sensitivity test) which would cause maximum elevations in cortisol levels could be ascertained. Basal blood samples were taken before and 15-20 min after the IV infusion of 0.5, 5, 25 or 50 units ACTH.

Perfusion Apparatus and Materials

The perfusion apparatus (Fig. 1) consisted of a specially constructed plastic block (L = 42 mm; H = 40 mm; W = 13 mm) with a bottom outlet which was connected to the cerebroventricular cannula for perfusion of normal or experimental CSF (Table I). An outlet on top of the block was connected via a Statham transducer to a polygraph for monitoring CSF pressure. The temperature of the CSF was maintained at 37.5°C by heating a nichrome wire surrounding the CSF inlet tube with approximately 70 volts from a variable transformer. The CSF temperature was monitored with a thermistor probe (Atkins Technical Inc.). The perfusion apparatus was needed to introduce normal or experimental CSF, which were contained in two beakers and agitated by magnetic stirrers, into the cerebroventricles via a micro-infusion pump (Holter; Model RL 175) at constant temperature (37.5°C), pressure (15 cm H_2O) and rate (50 µl/min).

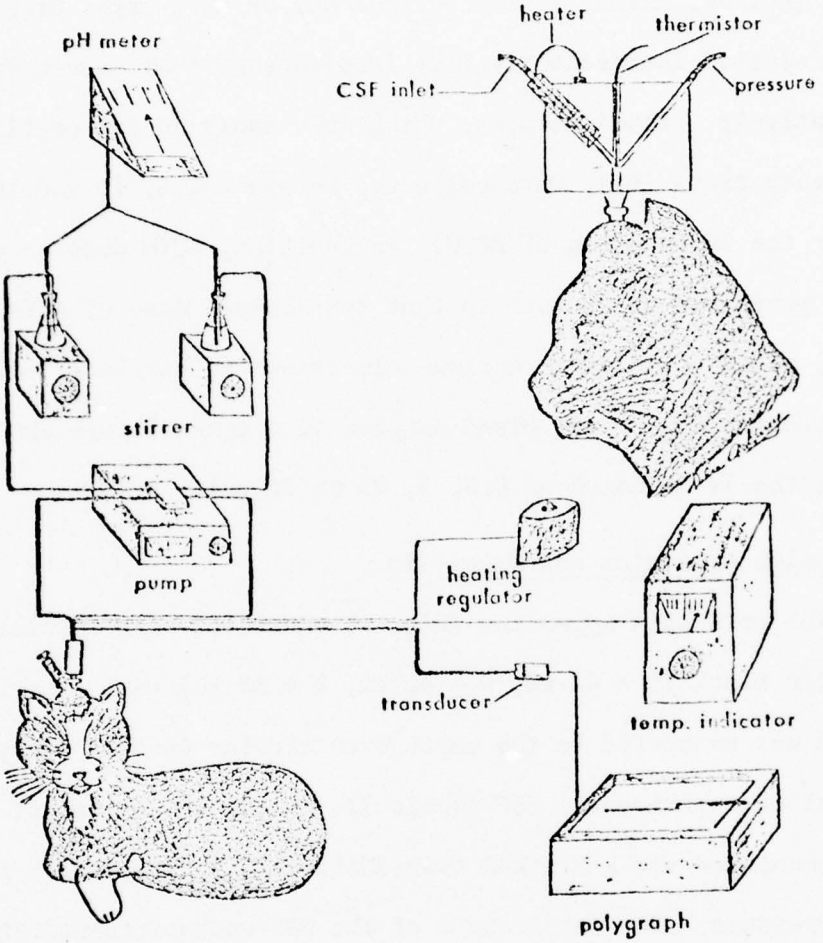


Figure 1. The perfusion apparatus and other equipment required to deliver experimental CSF at 37.5°C and 50 µl/min while maintaining CSF pressure, temperature and pH.

TABLE 1

CSF SOLUTIONS USED FOR PERFUSING THE CEREBROVENTRICLES OF CATS

Groups	CSF composition (mEq/L)[a]							
	Na^+	K^+	Li^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Urea	Glucose
Normal	158	2.98	0.00	3.00	1.33	24.6	6.70	3.68
Sodium								
High	168	2.98	0.00	3.00	1.33	24.6	0.00	0.00
Low	148	2.98	0.00	3.00	1.33	24.6	13.00	7.00
Potassium								
High	152	9.00	0.00	3.00	1.33	24.6	6.70	3.68
Low	161	0.00	0.00	3.00	1.33	24.6	6.70	3.68
Lithium								
High	156	2.98	2.00	3.00	1.33	24.6	6.70	3.68
Low	157	2.98	1.00	3.00	1.33	24.6	6.70	3.68
Calcium								
High	152	2.98	0.00	9.00	1.33	24.6	6.70	3.68
Low	161	2.98	0.00	0.00	1.33	24.6	6.70	3.68
Magnesium								
High	155.3	2.98	0.00	3.00	3.99	24.6	6.70	3.68
Medium	158.7	2.98	0.00	3.00	0.67	24.6	6.70	3.68
None	159.3	2.98	0.00	3.00	0.00	24.6	6.70	3.68

[a]Osmolality (314.8 mOsm) kept constant by varying concentrations of Na^+, urea or glucose. The pH was adjusted to 7.35 with 3 N HCl.

The composition of normal mock CSF (Table I) for cats was formulated according to published reports (2, 7, 22, 54). Since previous experiments (83, 84) from our laboratory have shown that altering CSF $[H^+]$ can markedly affect the cortisol secretion of dogs, the $[H^+]$ was rigorously controlled at pH 7.35 in both normal and experimental CSF. In addition, the osmolality was maintained at 314.8 mOsm by varying primarily the urea and glucose content.

Perfusion Procedure

On the morning of experimentation, the animals (seven days post-operative) were brought into the testing room, a quiet location adjoining the main laboratory, where they were kept for at least one hour prior to the beginning of experimentation. The perfusion apparatus was locked into the ventricular cannula (Fig. 1) and the animal placed in an open top box (46x46x46 cm). Perfusion of cats with normal CSF at 50 µl/min was then started (15, 22). The CSF pressure was recorded on a Grass polygraph. Those animals whose CSF pressure increased or did not stabilize at normal CSF pressure (10-20 cm H_2O) were immediately excluded from further perfusion. After 30 min a control or pre-period blood sample was collected. Immediately following the collection of the control blood sample, the polyvinyl tube carrying normal CSF was replaced with the experimental CSF line and the cerebroventricles were perfused for an additional 60 min with blood samples being taken at 30 and 60 min. To ascertain the maximal responsiveness of the adrenal cortex, at the end of each experimental period 20-25 units ACTH were infused over two minutes into the external jugular vein, the cannula flushed with saline and a blood sample taken approximately 20 min

later. All blood samples were analyzed for cortisol fluorometrically (64).

Perfusion Experiments

1. Effects of the Perfusion Apparatus and Anesthesia

To further test the experimental model, it was necessary to determine the effects of the perfusion apparatus mounted on the conscious animals on plasma cortisol levels and the tolerance of the cat to this procedure. Three groups of cats were used in this study. One group of cats (six trials on six cats) did not have the perfusion apparatus attached to the ventricular cannula. A second group (six trials on six cats) had the apparatus attached without perfusing CSF and a third group (six trials on five cats) had the apparatus attached with normal CSF perfused at 50 µl/min through the ventricles. Blood samples were collected at 30 min (control or pre-period), 60 and 90 min (30 and 60 min experimental periods) and at 110 min (ACTH period).

Anesthesia experiments were also performed in order to determine whether the conscious cat would be a better animal model for perfusion with CSF and not result in significant changes in basal plasma cortisol levels. Cats were anesthetized with sodium pentobarbital (25 mg/kg; Abbott Laboratories) and perfused with normal CSF for 90 min with 20 units ACTH being infused IV at the end of experimental period while normal CSF was perfused for an additional 20 min.

2. CSF Cation Studies

A series of experiments were designed to ascertain whether

altered CSF cation concentrations known to occur during various environmental stressors, such as hypoxia and hypercapnia (9), would affect the HHA system. Various concentrations of CSF Na^+, K^+, Ca^{2+} and Mg^{2+} (Table I), within physiological ranges, were perfused (50 µl/min) through the cerebroventricles of conscious cats. In two groups of animals 1.0 and 2.0 mEq Li^+/L were substituted for Na^+. Following a 30 min control period during which the animal was perfused with normal CSF, increased or decreased amounts of the above mentioned cations (73 trials on 27 cats) were perfused during a 60 min experimental period with ACTH infused IV at the end of this period.

3. Agonist and Antagonists Studies

In order to separate the α- and β-receptor actions of norepinephreine and the n- and m-receptor actions of acetylcholine on HHA activity, the α-adrenergic blocker, phentolamine (Ciba Pharm. Co.), the β-blocker, propranolol (Sigma Chemical Co.), the n-blocker, mecamylamine (Sigma Chemical Co.) and the m-blocker, atropine sulfate (Mallinchrodt Chemical Works), were added to normal CSF and perfused through the cerebroventricles of conscious cats (71 trials on 33 cats). Selection of doses and perfusion rates for the α- and β-blockers were based on those of Heise and Kroneberg (50, 51), who perfused the ventricles of dogs with 30 and 10 µg/min, respectively. In addition, the doses were modified in order to avoid overt behavioral effects such as vocalization, tremors and excitability when conscious cats were perfused with the various blockers. Thus, a group of cats (four trials on four cats) was

perfused with phenotolamine at 30 µg/min and a second group (six trials on five cats) with 1.0 µg/min, while propranolol was perfused at 10 µg/min (four trials on four cats) and 0.3 µg/min (nine trials on seven cats). Another group of cats was perfused simultaneously with phentolamine (1.0 µg/min) and propranolol (0.3 µg/min) in order to determine the effect of blocking both α- and β-receptors on HHA activity.

Mecamylamine was perfused at 0.8 µg/min (seven trials on six cats) and 3.0 µg/min (seven trials on seven cats), while atropine was first perfused at 0.8 µg/min (seven trials on five cats) and then at 3.0 µg/min (eight trials on six cats). Perfusion doses and rates were obtained from Ganong (43) who perfused the cerebroventricles of surgically stressed dogs with atropine. These low doses of atropine were selected since it is known that high doses affect both n- and m-receptors, whereas low doses affect primarily the m-receptors (54). Mecamylamine together with atropine was perfused (six trials on four cats) at 0.8 µg/min in another group of experiments. In order to determine the interaction of both adrenergic and cholinergic receptors on HHA activity, phentolamine (1.0 µg/min), propranolol (0.3 µg/min), mecamylamine (0.8 µg/min) and atropine (0.8 µg/min) were combined and perfused in six cats (six trials).

4. GABA Studies

The influence of the hippocampus (44) on adrenocortical activity and the presence of GABA (126) in this limbic structure prompted the perfusion of GABA in order to ascertain the effects

of this neurotransmitter on HHA activity. From the perfusion studies performed on rats by Makara and Stark (82), the dose and perfusion rate of GABA were determined. GABA (Sigma Chemical Co.) was added to normal CSF and perfused (100 µg/min) in six cats (six trials). Picrotoxin, a γ-blocker for GABA, was perfused in order to further delineate the action of GABA on HHA activity. The dose and perfusion rate of picrotoxin (Sigma Chemical Co.) was obtained from the studies of Lee and Yang (80) and from preliminary experiments in which various doses of the blocker were added to normal CSF. Behavioral effects were the primary factors that resulted in the selection of 1.0 µg picrotoxin/min to perfuse through the ventricles of five cats (five trials). In order to ascertain the interaction of GABA and picrotoxin, the neurotransmitter and antagonist were perfused simultaneously in four cats (four trials) at 100 µg/min and 1.0 µg/min, respectively.

Another group (seven trials on seven cats) of experiments included the simultaneous perfusion of phentolamine (1.0 µg/min), propranolol (0.3 µg/min), mecamylamine (0.8 µg/min), atropine (0.8 µg/min) and picrotoxin (1.0 µg/min) in order to ascertain the effects of the interaction of the adrenergic, cholinergic and gabanergic neural systems on plasma cortisol levels.

5. Cation and Neurotransmitter Study

In order to ascertain whether the effect of CSF cations (e.g., Ca^{2+} lack) on HHA activity was mediated through neurotransmitters (e.g., adrenergic), CSF containing norepinephrine (0.1 ng/ml) and a lack of Ca^{2+} was selected for perfusion through the cerebroventricles

of two cats (five trials). The perfusion rate of norepinephrine was obtained from the cerebroventricular perfusion studies of Van Loon et al., (129).

6. Dexmethasone Studies

Dexamethasone (Sigma Chemical Co.) was added to CSF and perfused through the ventricles in order to determine whether this synthetic glucocorticoid was capable of inhibiting the stimulatory effects of various cations and agonists-antagonists. To perform these studies it was first necessary to ascertain the dose-perfusion rate of dexamethasone which would lower, if possible, the basal cortisol levels in the cat model. The dose and perfusion rate was determined by noting the effects of various rates (2-25 µg/min) which caused minimal behavioral changes while still lowering plasma cortisol levels. Dexamethasone was added to normal CSF and perfused (25 µg/min) in six cats for 90 min prior to being injected IV with ACTH. Once this dose had been established, dexamethasone was added to CSF containing no K^+ and then perfused through the ventricles of five cats (six trials) for 80 min after first perfusing the ventricles for 30 min with dexamethasone added to normal CSF. Similar experiments were performed by adding dexamethasone to CSF containing 1) no Ca^{2+} (six trials on five cats); 2) GABA (100 µg/min; six trials on three cats); or 3) the five blockers (phentolamine at 1.0 µg/min, propranolol at 0.3 µg/min, mecamylamine at 0.8 µg/min, atropine at 0.8 µg/min and picrotoxin at 1.0 µg/min).

7. Cortisol Determination and Calculation of Data

All plasma samples, which had been stored at -20°C, were

analyzed fluorometrically for cortisol according to the method of Kitabchi and Kitchell (64) with the exception that 2.0 ml rather than 1.0 ml fluorescent reagent was used. In order to ascertain whether the fluorometric method was indeed measuring cortisol, it was compared to a radioimmunoassay (34) in samples obtained from the ACTH-dose response study. These data showed that during basal periods the cortisol values by the radioimmunoassay were approximately 75% of the fluorometric method, whereas after the administration of ACTH the values were essentially similar. Based upon these results, the more economical and less time consuming fluorometric procedure was selected.

Since variations in the pre-period (control) levels of plasma cortisol were observed among the cats, the data from the perfusion studies are reported as the percent (%) change in cortisol levels for each cat from the pre-period value. Group means were calculated from these % values and are expressed in the text as % cortisol changes. Statistical significance was ascertained by the independent t-test.

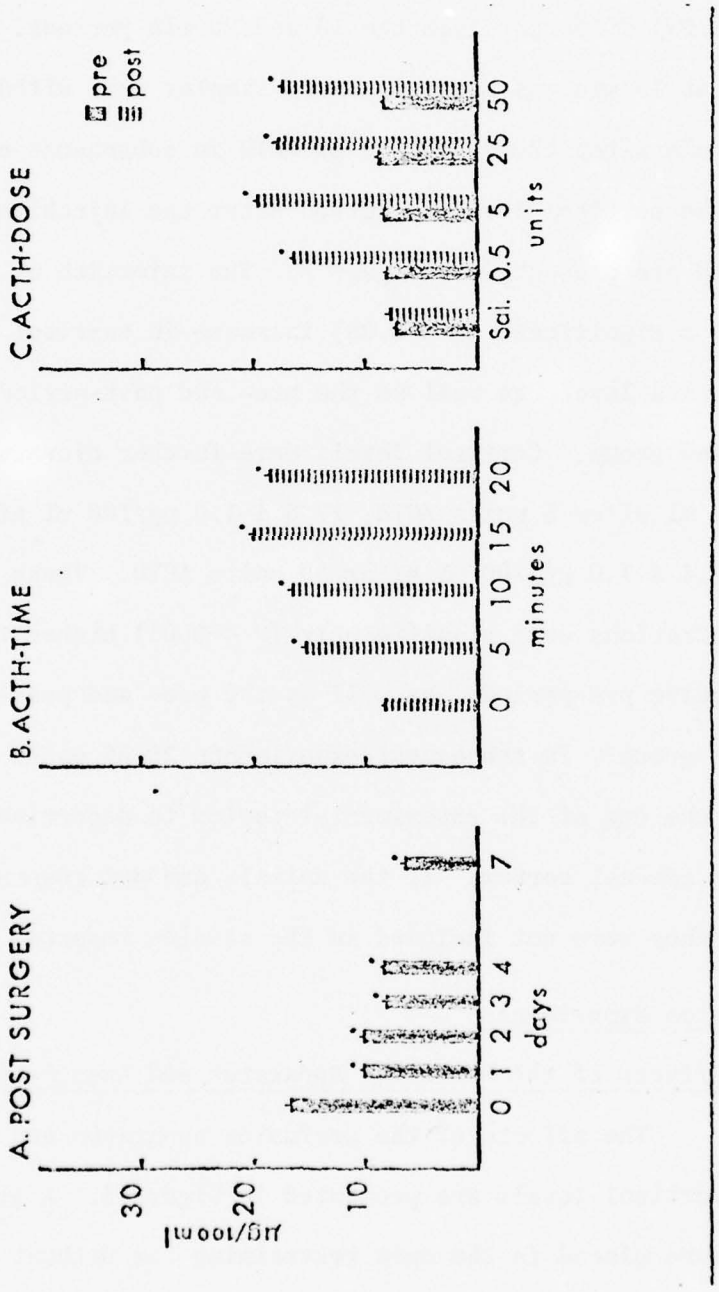
III. RESULTS

Validation of the Animal Model

The effect of the surgical procedure on the plasma cortisol levels of cats immediately following surgery and on days 1, 2, 3, 4 and 7 is illustrated in Figure 2. The mean cortisol level after surgery was 16.8 ± 1.4 µg/100 ml and decreased significantly ($P < 0.05$) to 10.2 ± 0.7 and 10.3 ± 1.2 µg/100 ml on days 1 and 2, respectively. On days 3 and 4 the cortisol levels further decreased to 8.3 ± 1.1 and 8.6 ± 0.8 µg/100 ml, respectively, and these were significantly lower ($P < 0.05$) than on the day of surgery but not from those noted on days 1 and 2. The further decrease to 6.6 ± 0.8 µg/100 ml on day 7 was significantly ($P < 0.05$) lower than the day of surgery as well as on the first two post-surgical days. Although cortisol levels decreased markedly after the first day of surgery, in all subsequent experiments at least 7 days were allowed for basal cortisol levels (72) to be attained and for the complete recovery of general health (e.g., appetite, drinking, etc.). In addition, seizure activity that occurred when cerebroventricular perfusions were performed shortly after surgery was almost completely eliminated by 7 days postsurgery.

Five min after the rapid injection of 20-25 units ACTH, cortisol levels (15.6 ± 1.3 µg/100 ml) increased significantly ($P < 0.05$) from those (8.3 ± 0.6 µg/100 ml) observed prior to the administration of ACTH (Fig. 2). Further increases in cortisol levels occurred at 10 (16.4 ± 1.4 µg/100 ml), 15 (20.1 ± 1.4 µg/100 ml) and 20 (18.5 ± 1.9

Figure 2. Plasma cortisol levels of 13 cats (40 blood samples) following surgery (A); six cats after the injection of 20-25 units ACTH (B); and 22 cats (25 trials) after the injection of various doses of ACTH (C). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from day of surgery (A), zero time (B) or pre-period (C).


A. POST SURGERY
B. ACTH-TIME
C. ACTH-DOSE
pre
post
μg/100ml
30
20
10
0 1 2 3 4 7
days
0 5 10 15 20
minutes
Sci. 0.5 5 25 50
units

μg/100 ml) min and these were significantly ($P < 0.05$) above those of their respective pre-injection periods. Comparisons of the cortisol levels among the 5, 10 and 20 min periods were not significant ($P > 0.05$); however, the steroid concentration at 15 min was significantly ($P < 0.05$) higher than at 5 min but not significantly ($P > 0.05$) different from the 10 and 20 min periods. Since the cortisol level at 15 min was maximal, blood samples were withdrawn between 15-20 min after the injection of ACTH in subsequent experiments.

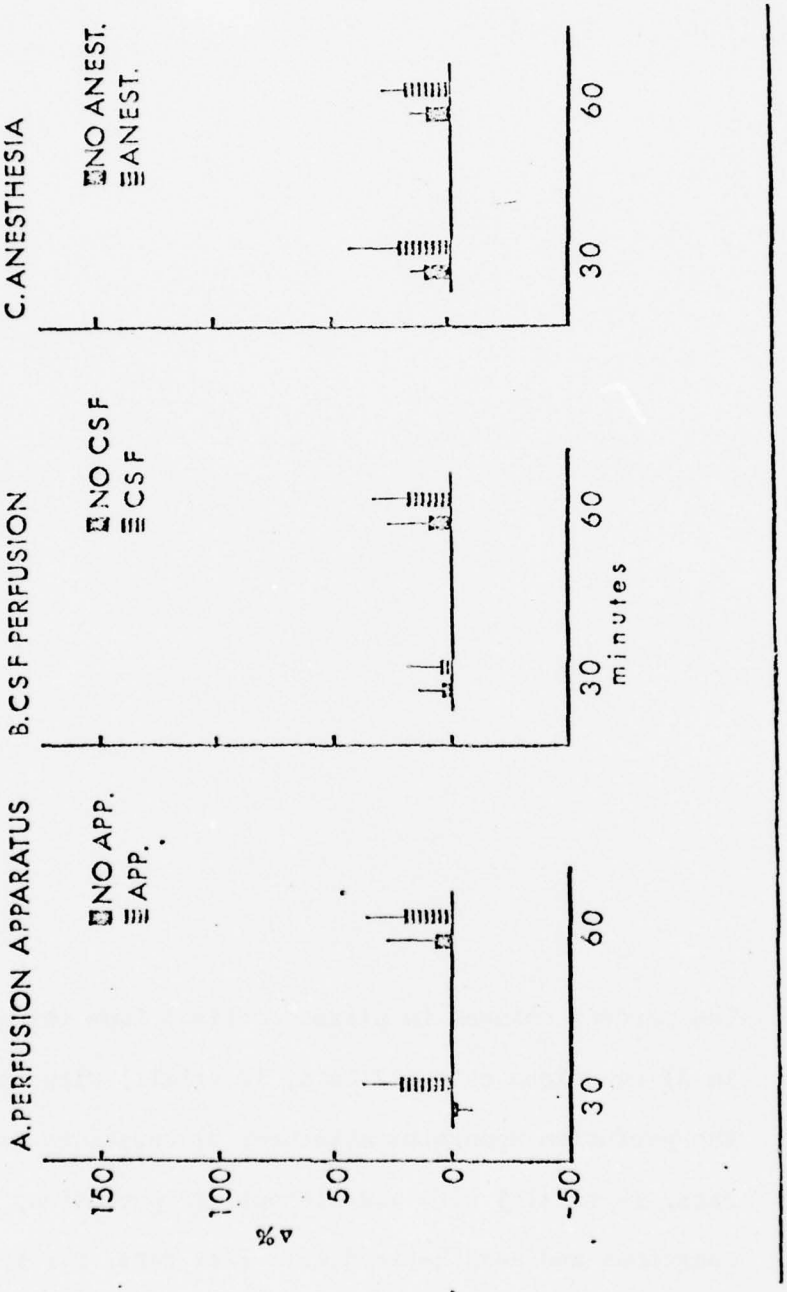
The cortisol levels observed after the injection of various doses of ACTH are presented in Figure 2. The injection of 0.5 unit ACTH caused a significant ($P < 0.05$) increase in cortisol level over its pre-period level, as well as the pre- and post-periods of the saline injected group. Cortisol levels were further elevated to 19.8 ± 1.0 μg/100 ml after 5 units ACTH, 17.8 ± 1.0 μg/100 ml after 25 units ACTH and 17.4 ± 1.0 μg/100 ml after 50 units ACTH. These cortisol concentrations were significantly ($P < 0.05$) higher than those of their respective pre-periods, as well as the pre- and post-periods of the saline group. In subsequent experiments 20-25 units ACTH were injected IV at the end of the experimental period to determine maximal stimulation of the adrenal cortex. If the animals did not respond to this dose of ACTH, they were not included in the studies reported herein.

Perfusion Experiments

1. Effects of the Perfusion Apparatus and Anesthesia

The effects of the perfusion apparatus and normal CSF on plasma cortisol levels are presented in Figure 3. A group of cats, which were placed in the open restraining box without the perfusion

Figure 3. The percent changes in plasma cortisol from the pre-period in A) conscious cats (12 cats, 12 trials) with and without the perfusion apparatus attached; B) conscious cats (11 cats, 12 trials) with and without CSF perfusion; and C) conscious and anesthetized cats (six cats, six trials) perfused with normal CSF. Bar with vertical line indicates the mean ± S.E.M.

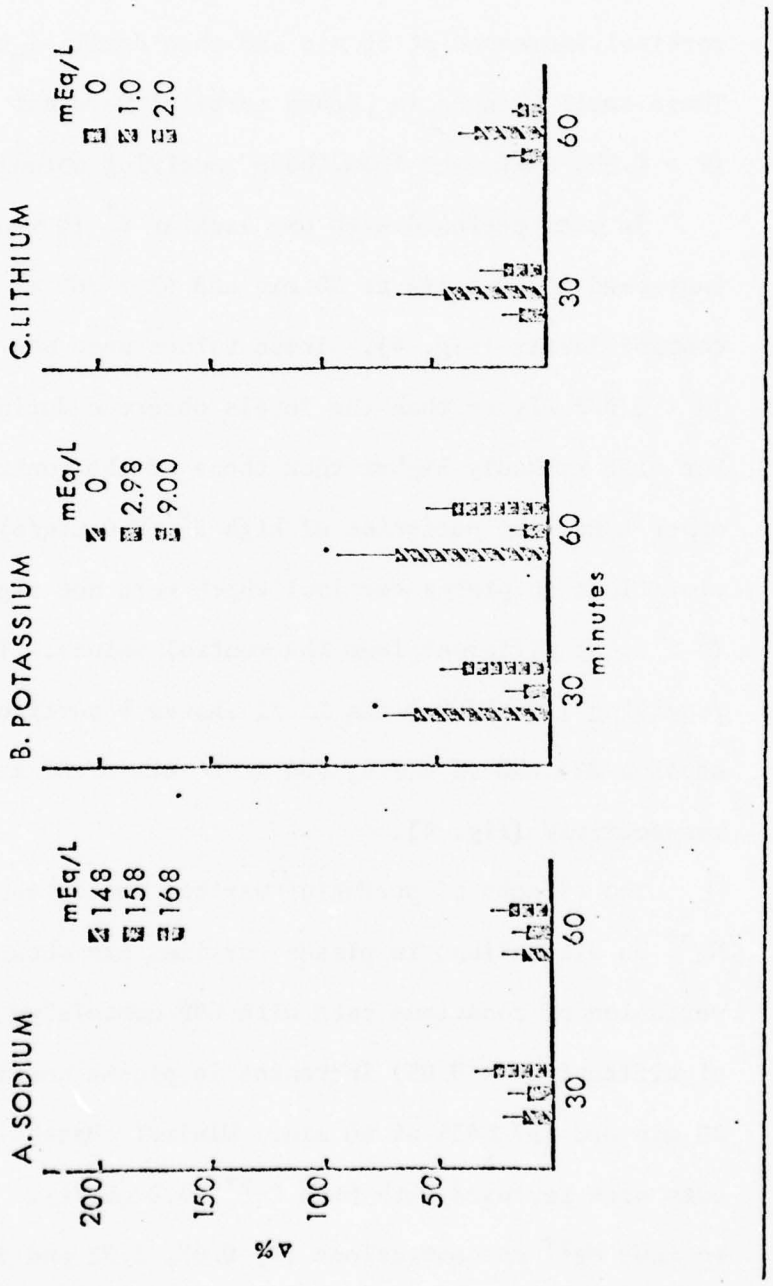

A. PERFUSION APPARATUS
NO APP.
APP.
B. CSF PERFUSION
NO CSF
CSF
C. ANESTHESIA
NO ANEST.
ANEST.
150
100
50
0
-50
% ∇
30
60
30
60
30
60
minutes

apparatus attached to the ventricular cannula, exhibited % cortisol changes during the 30 (-2 ± 7%) and 60 (7 ± 20%) min experimental periods which were not significantly ($P > 0.05$) different from the control or pre-period values. In another group of cats with the perfusion apparatus attached to the ventricular cannula, plasma cortisol during the 30 (22 ± 16%) and 60 (21 ± 16%) min periods did not vary significantly ($P > 0.05$) from one another or from the group without the apparatus attached at comparable periods. In cats with the apparatus attached to the ventricular cannula and normal CSF perfused through the ventricles, the cortisol changes during the 30 and 60 min periods were 4 ± 14% and 18 ± 15%, respectively, above the pre-period. These values were not significantly ($P > 0.05$) higher than those of the group without the apparatus attached for the same period or the cats with the apparatus attached but without perfusing CSF. Figure 3 also shows the % cortisol changes for the 30 and 60 min periods of anesthetized cats perfused with normal CSF. These cortisol changes were not significantly ($P > 0.05$) different from those of conscious cats perfused with CSF. Since perfusion of anesthetized cats with normal CSF offered no advantages in terms of basal cortisol levels over the conscious cat model, the latter was selected as being the more physiological preparation.

2. Cation Studies

Presented in Figure 4 are the % cortisol changes from the pre-periods caused by the perfusion of various concentrations of Na^+, K^+ and Li^+. The cortisol values for both the 30 and 60 min

Figure 4. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with CSF containing various concentrations of A) sodium (18 cats, 19 trials); B) potassium (13 cats, 13 trials); and C) lithium (11 cats, 11 trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from control group (solid bar).

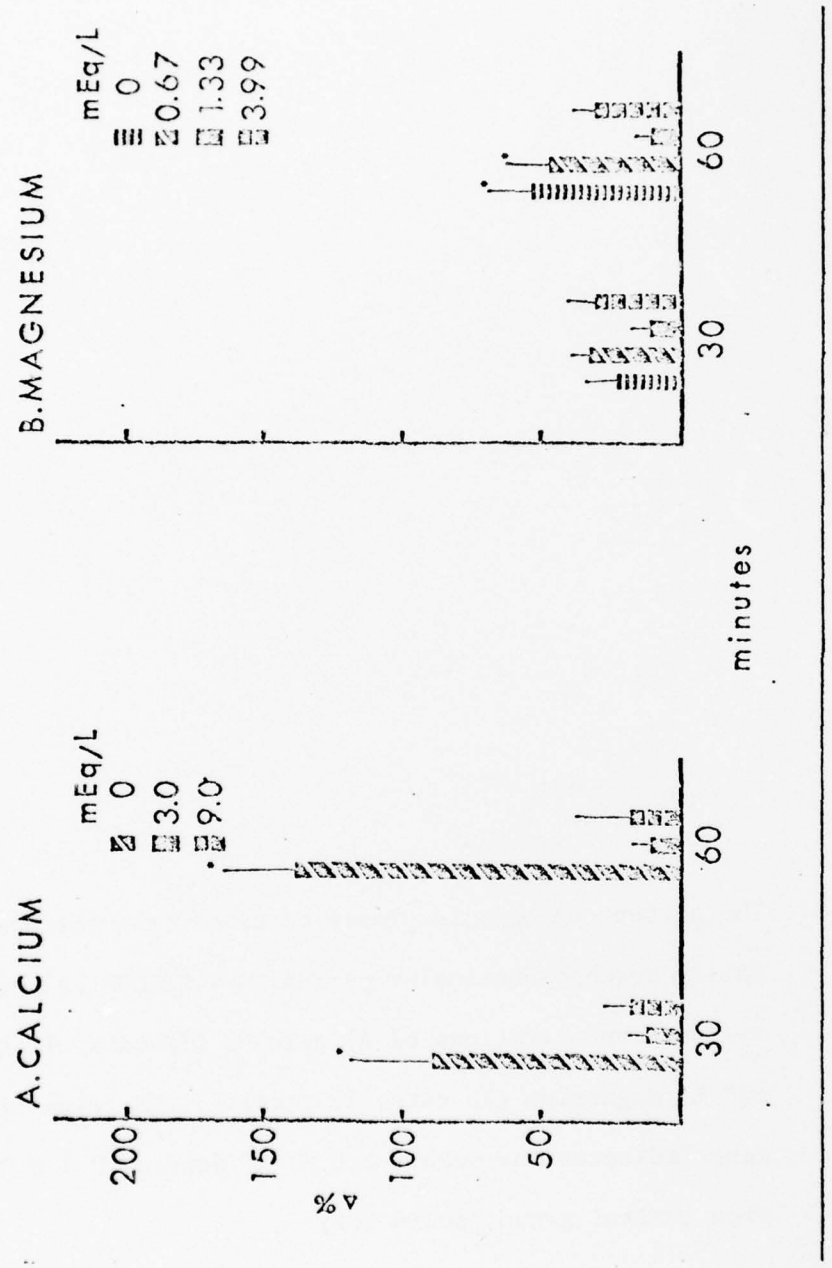

A. SODIUM
mEq/L
148
158
168
B. POTASSIUM
mEq/L
0
2.98
9.00
C. LITHIUM
mEq/L
0
1.0
2.0
200
150
100
50
Δ%
30
60
minutes

periods of the low Na^+ (148 mEq/L) group were not significantly ($P > 0.05$) different from those observed at 30 (11 ± 8%) and 60 (10 ±7%) min in the control group which was perfused with normal quantities of Na^+ (158 mEq/L). In the high Na^+ (168 mEq/L) group, cortisol increased at 30 min and then declined slightly at 60 min. These small changes in plasma cortisol were not significantly ($P > 0.05$) different from those receiving normal quantities of Na^+.

In cats perfused with CSF lacking K^+ (0 mEq/L) plasma cortisol increased to 60 ± 14% at 30 min and 67 ± 26% at 60 min above control levels (Fig. 4). These values were not only significantly ($P < 0.05$) higher than the levels observed during the pre-period but also markedly higher than those of the control group. On the other hand, the perfusion of high K^+ (9.0 mEq/L) caused modest elevations in plasma cortisol which were not significantly ($P > 0.05$) different from the control values. The two groups receiving 1.0 and 2.0 mEq Li^+/L showed % cortisol changes at 30 min of 45 ± 22% and 18 ± 11%, and at 60 min of 32 ± 8% and 12 ± 5%, respectively (Fig. 4).

The effects of perfusing various concentrations of Ca^{2+} and Mg^{2+} on alterations in plasma cortisol are shown in Figure 5. The perfusion of conscious cats with CSF containing no Ca^{2+} caused significant ($P < 0.05$) increases in plasma cortisol of 89 ± 29% at 30 min and 137 ±43% at 60 min. Minimal changes were observed when cats were perfused with high Ca^{2+} (9.0 mEq/L). Cats perfused with various Mg^{2+} concentrations (0, 0.67, 1.33 and 3.99 mEq/L) showed modest elevations in plasma cortisol; however, only the lower

Figure 5. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with CSF containing various concentrations of A) calcium (12 cats, 12 trials); and B) magnesium (15 cats, 18 trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from control group (solid bar).


A.CALCIUM
mEq/L
0
3.0
9.0
200
150
100
50
Δ%
30
60
B.MAGNESIUM
mEq/L
0
0.67
1.33
3.99
30
60
minutes

concentrations of Mg^{2+} at 60 min caused significant ($P < 0.05$) elevations above the control group.

All cats perfused with various concentrations of monovalent and divalent cations exhibited increases of 80% to 112% when ACTH was administered IV at the end of the experimental period (Table II).

3. Agonist and Antagonist Studies

Animals perfused with normal CSF containing the α-adrenergic blocker, phentolamine (1.0 µg/min) showed increases ($P < 0.05$) in plasma cortisol above the pre-period of 49 ± 16% at 30 min and 66 ± 18% at 60 min (Fig. 6). The plasma cortisol of four cats perfused with phentolamine at 30 µg/min increased markedly ($P < 0.05$) at 30 (83 ± 38%) and 60 (219 ± 54%) min. These experiments had to be aborted since conscious cats were not able to withstand this dose without exhibiting many behavioral (e.g., restlessness, vocalization, etc.) and physiological (e.g., defecation, salivation, etc.) changes. The plasma cortisol of cats perfused with normal CSF containing the β-adrenergic blocker, propranolol (0.3 µg/min), increased though not significantly ($P > 0.05$) 31.5 ± 5% at 30 min, while at 60 min the 49 ± 10% increase in plasma cortisol was significantly ($P < 0.05$) higher than the control group (Fig. 6). Four cats perfused at 10 µg/min exhibited significant ($P < 0.05$) changes in cortisol levels of 136 ± 49% at 30 min and 272 ± 51% at 60 min. Additional animals were not perfused with propranolol at this high dose because of similar behavioral and physiological changes that occurred with the high dose of phentolamine. When cats were perfused with normal

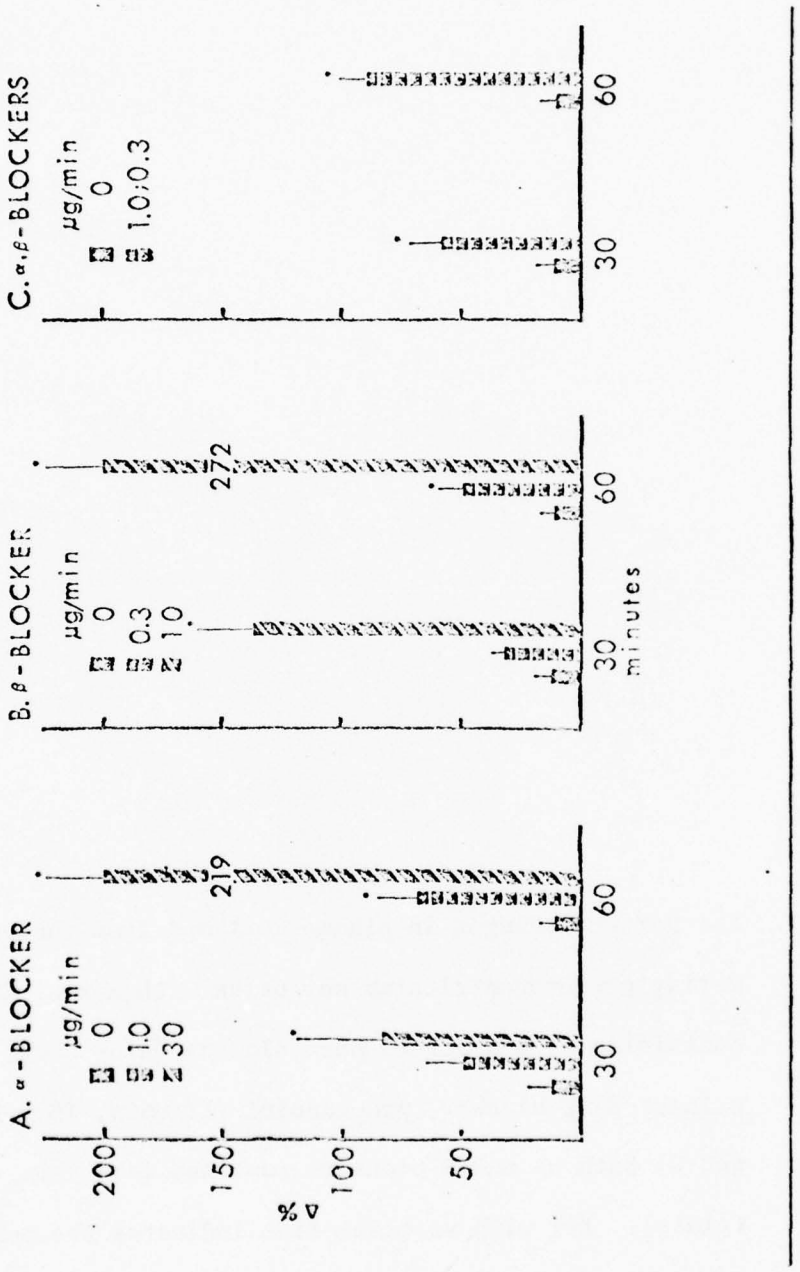
TABLE II

PERCENT CHANGE FROM PRE-PERIOD IN PLASMA CORTISOL CAUSED BY ACTH (20 units) ADMINISTERED IV AT THE END OF THE EXPERIMENTAL PERIOD IN CATS PERFUSED WITH CSF CONTAINING VARIOUS CONCENTRATION OF CATIONS

Cation mEq/L	Cortisol Δ%
Sodium	
148	80 ± 10[a]
158	100 ± 19
168	105 ± 10
Potassium	
0	112 ± 19
9.0	104 ± 14
Lithium	
1.0	106 ± 16
2.0	111 ± 24
Magnesium	
0	84 ± 15
0.67	93 ± 15
3.99	112 ± 25
Calcium	
0	108 ± 16
9.0	100 ± 16

[a]Mean ± S.E.M.

Figure 6. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with normal CSF containing A) α-blocker, phentolamine (nine cats, ten trials; B) β-blocker, propranolol (11 cats, 13 trials); and C) both α- and β-blockers combined (six cats, seven trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from control group (solid bar)


A. α-BLOCKER
μg/min
0
1.0
30
B. β-BLOCKER
μg/min
0
0.3
1.0
C. α,β-BLOCKERS
μg/min
0
1.0;0.3
219
272
200
150
100
50
Δ %
30
60
minutes

CSF containing the lower doses of both phentolamine (1.0 µg/min) and propranolol (0.3 µg/min), plasma cortisol increased significantly ($P < 0.05$) at both 30 (58 ± 14%) and 60 (89 ± 12%) min (Fig. 6). These cortisol values were higher than those noted when the two blockers were perfused alone for the same duration.

Figure 7 illustrates the % cortisol changes observed when the cerebral ventricles of cats were perfused with cholinergic antagonists. In animals perfused with mecamylamine (n-blocker) at 0.8 µg/min, the changes observed in cortisol at 30 and 60 min were not significantly ($P > 0.05$) different from the control group. When the perfusion of cats with mecamylamine was increased to 3.0 µg/min, plasma cortisol levels were neither significantly ($P > 0.05$) different from the pre-period values nor from the control group. The perfusion of cats with atropine (m-blocker) at 0.8 or 3.0 µg/min did not cause significant ($P > 0.05$) alterations in plasma cortisol levels for the 30 or 60 min periods. In addition, combining mecamylamine (0.8 µg/min) and atropine (0.8 µg/min) in the perfusion solution did not significantly ($P > 0.05$) alter plasma cortisol throughout the experimental period.

The effects of perfusing GABA (100 µg/min) on plasma cortisol are presented in Figure 8. A 49 ± 12% increase ($P < 0.05$) from the pre-period was observed at 30 min which continued to rise to 69 ± 12% ($P < 0.05$) at 60 min. On the other hand, perfusion of cats with the γ-blocker, picrotoxin (1.0 µg/min), did not alter steroid levels. When animals were perfused with a combination of GABA (100 µg/min) and picrotoxin (1.0 µg/min), the cortisol values

Figure 7. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with normal CSF containing A) the n-blocker, mecamylamine (13 cats, 14 trials); B) m-blocker, atropine (11 cats, 15 trials); and C) both n- and m-blockers combined (four cats, six trials). Bar with vertical line indicates the mean ± S.E.M.
* denotes $P < 0.05$ from control group (solid bar).

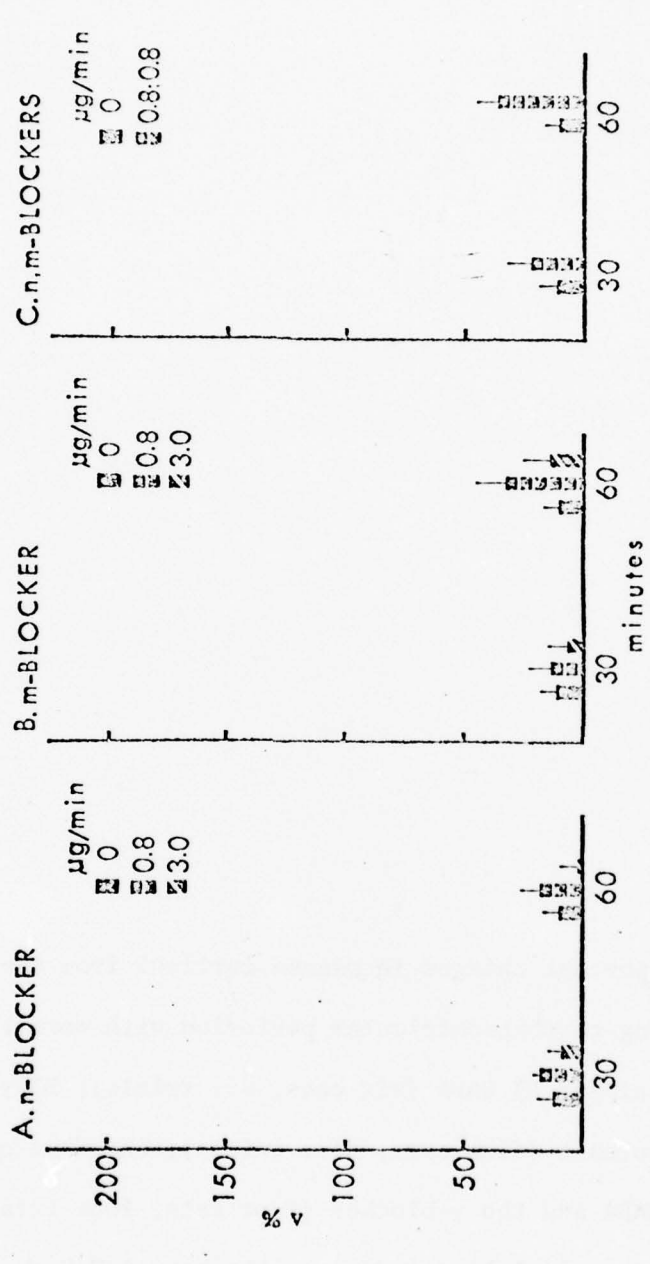
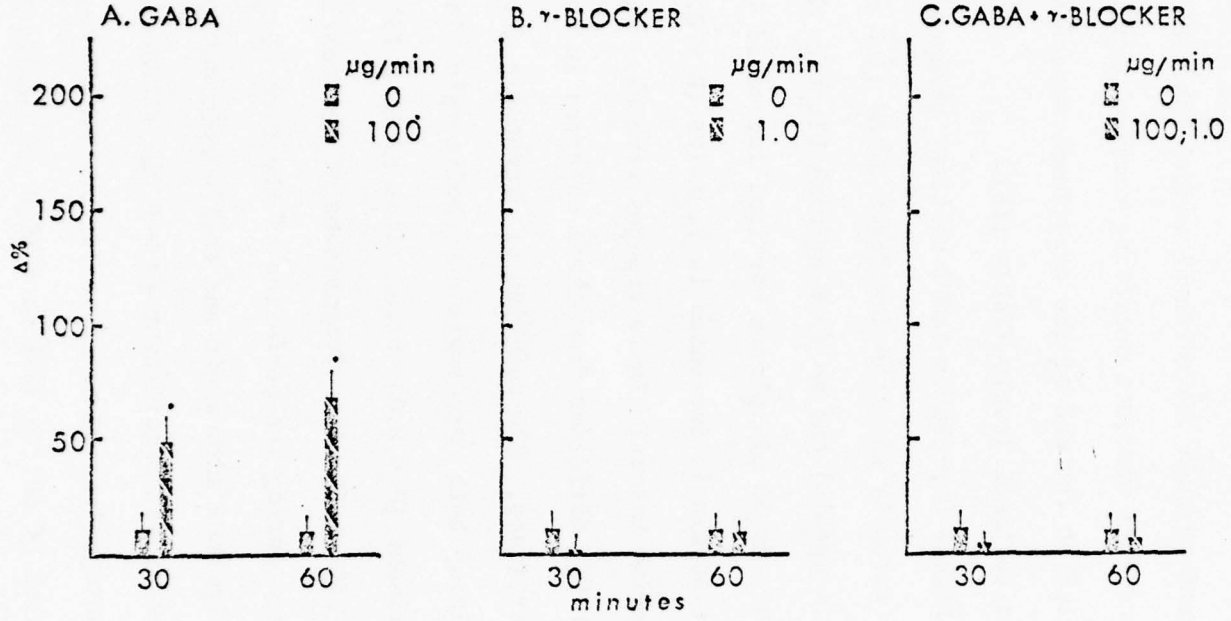

A.n-BLOCKER
B.m-BLOCKER
C.n,m-BLOCKERS
µg/min
0
0.8
3.0
0.8:0.8
200
150
100
50
% v
30
60
minutes

Figure 8. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with normal CSF containing A) GABA (six cats, six trials); B) γ-blocker, picrotoxin (five cats, five trials); and C) a combination of GABA and the γ-blocker (four cats, four trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from the control group (solid bar).


A. GABA
B. γ-BLOCKER
C. GABA + γ-BLOCKER
μg/min
0
100
1.0
100;1.0
200
150
100
50
Δ%
30
60
minutes

at 30 (4 ± 4%) and 60 (6 ± 12%) min were similar to the pre-period levels. Furthermore, it is obvious that picrotoxin significantly ($P < 0.05$) inhibited the effect observed when GABA alone was perfused through the cerebral ventricles. GABA plus picrotoxin treated cats were not secreting cortisol maximally, since ACTH injected at the end of the experiment caused modest elevations in plasma cortisol levels (Table III).

Figure 9 presents the % cortisol changes of animals perfused simultaneously with the low doses of α- (1.0 µg/min), β- (0.3 µg/min), n- (0.8 µg/min) and m- (0.8 µg/min) blockers during the experimental period. At 30 min plasma cortisol increased 43 ± 10% ($P < 0.05$), and at 60 min it increased to 71 ± 10% ($P < 0.05$) above the pre-period values. These changes were also markedly ($P < 0.05$) different from those observed in the control group at similar times. The addition of picrotoxin (1.0 µg/min) to CSF containing both adrenergic and cholinergic blockers caused significant ($P < 0.05$) alterations of 53 ± 15% at 30 min and 110 ± 38% at 60 min. Comparisons of the cortisol values, which were observed during the perfusion of the five blockers with those noted when the four adrenergic and cholinergic antagonists were perfused, did not reveal significant ($P > 0.05$) differences. Cats perfused with antagonists alone or in various combinations exhibited increases of 69% to 233% when ACTH was administered IV at the end of the experimental period (Table III).

4. Cation and Neurotransmitter Study

The effects of perfusing cats with CSF containing norepinephrine (0.1 mg/ml) and a lack of Ca^{2+} are shown in Figure 10.

TABLE III

PERCENT CHANGE FROM PRE-PERIOD IN PLASMA CORTISOL CAUSED BY ACTH (20 units) ADMINISTERED IV AT THE END OF THE EXPERIMENTAL PERIOD IN CATS PERFUSED WITH CSF CONTAINING VARIOUS AGONISTS AND ANTAGONISTS

Drugs μg/min	Cortisol Δ%
α-blocker	
1.0	119 ± 57[a]
3.0	189 ± 56
β-blocker	
0.3	101 ± 38
10	233 ± 70
α, β-blockers	
1.0; 0.3	148 ± 56
γ-blocker	
1.0	87 ± 17
GABA	
100	123 ± 13
γ-blocker + GABA	
1.0; 100	69 ± 12
n-blocker	
0.8	106 ± 13
3.0	68 ± 6
m-blocker	
0.8	100 ± 10
3.0	69 ± 13
n, m-blockers	
0.8; 0.8	77 ± 14
α, β, n, m-blockers	
1.0; 0.3; 0.8; 0.8	107 ± 33
α, β, γ, n, m-blockers	
1.0; 0.3; 1.0; 0.8; 0.8	146 ± 87

[a]Mean ± S.E.M.

Figure 9. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with normal CSF containing A) the α, β, n, m-blockers (six cats, six trials; and B) the four blockers with the addition of the γ-blocker, picrotoxin (seven cats, seven trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from the control group (solid bar).

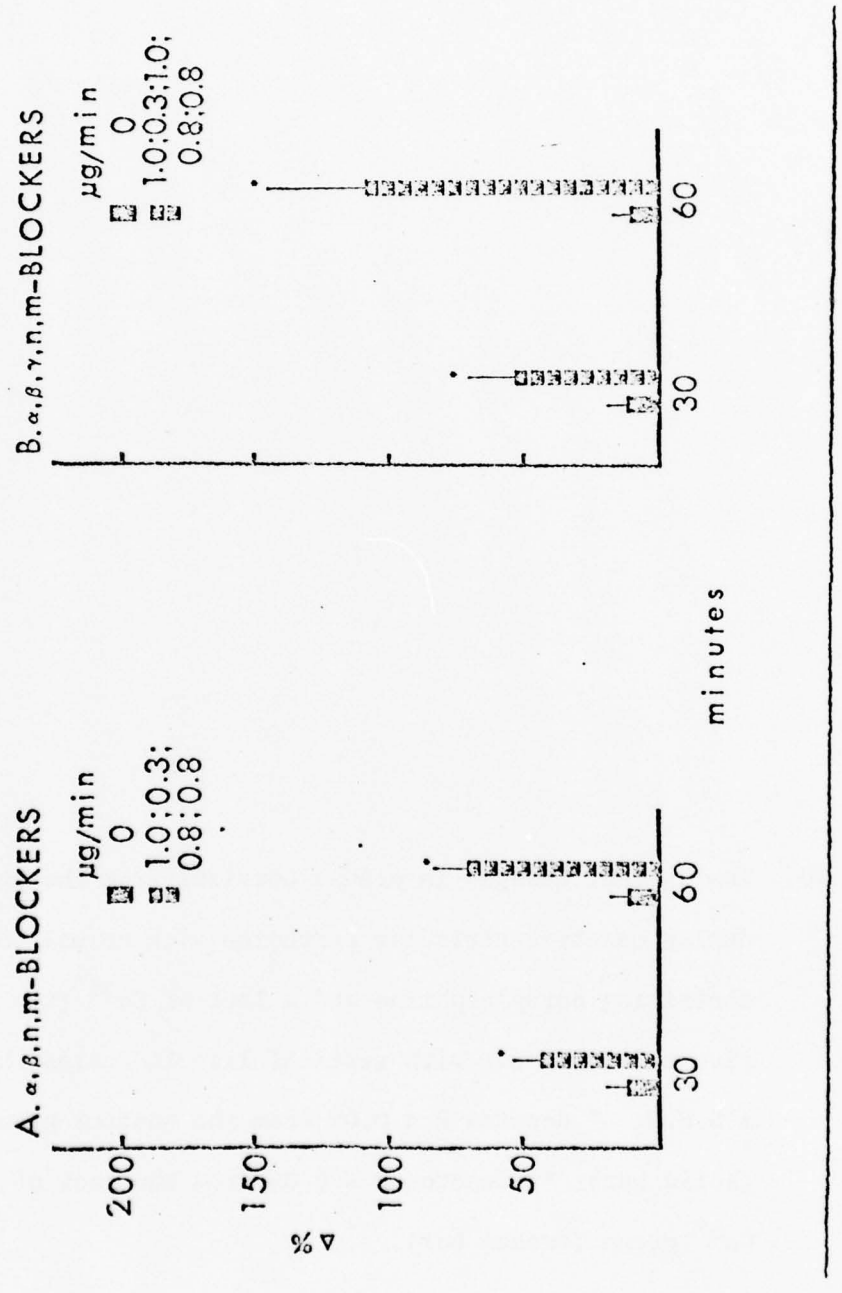
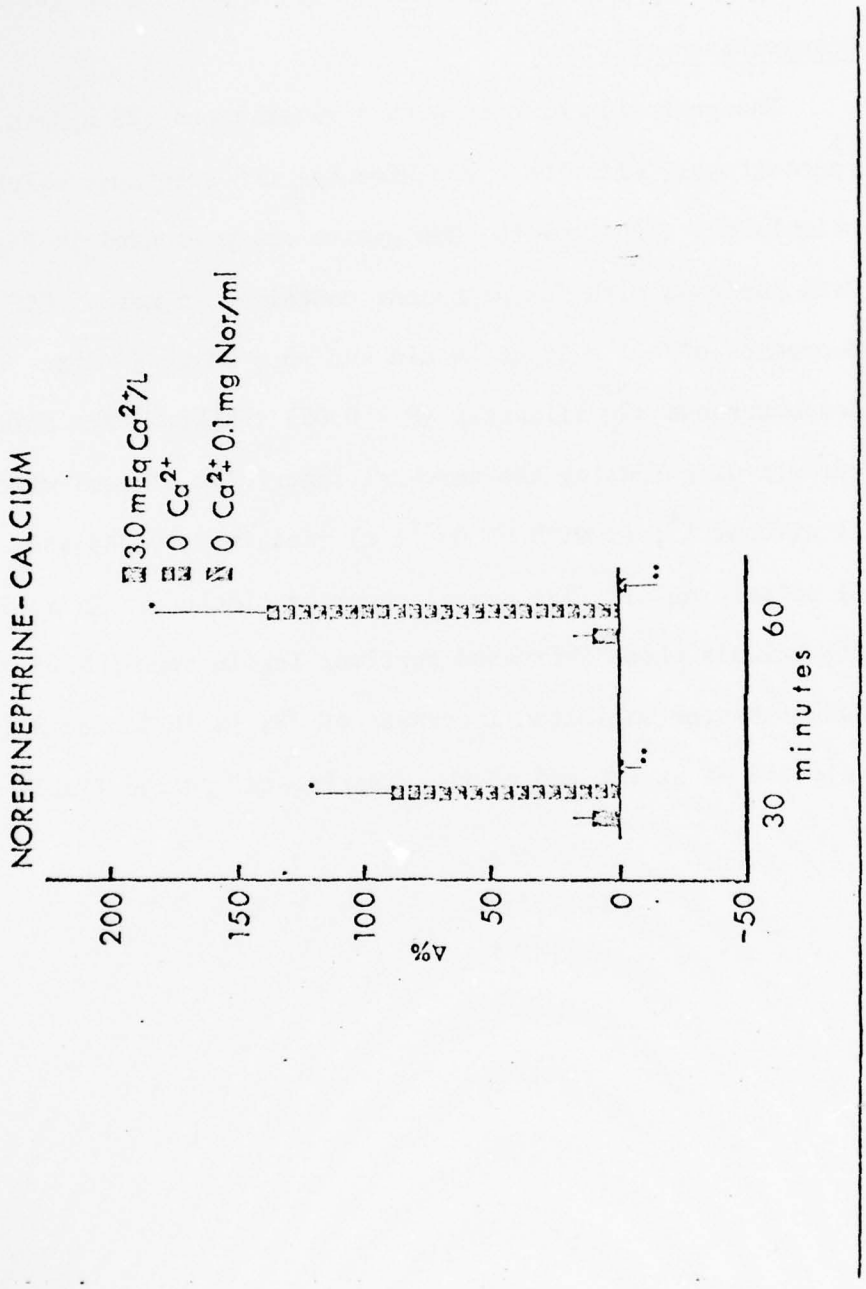

A. α,β,n,m-BLOCKERS
B. α,β,γ,n,m-BLOCKERS
μg/min
0
1.0:0.3;
0.8;0.8
1.0:0.3:1.0;
0.8;0.8
200
150
100
50
Δ%
30
60
minutes

Figure 10. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with normal CSF containing norepinephrine and a lack of Ca^{2+} (two cats, five trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from the control group (solid bar); ** denotes $P < 0.05$ from the lack of CSF Ca^{2+} group (broken bar).


NOREPINEPHRINE-CALCIUM
3.0 mEq Ca^{2+}/L
0 Ca^{2+}
0 Ca^{2+} 0.1 mg Nor/ml
200
150
100
50
0
-50
Δ%
30
60
minutes

Norepinephrine significantly ($P < 0.05$) inhibited the stimulatory action of a lack of CSF Ca^{2+} at 30 (-1 ± 8%) and 60 (-3 ± 12%) min.

5. Dexamethasone Studies

The perfusion of cats with dexamethasone (25 µg/min) concomitantly with those experimental CSF solutions which had a stimulatory effect on the HHA system are presented in Figure 11. Cats perfused with dexamethasone contained in normal CSF caused decreases of -11 ± 5% at 30 min and -8 ± 8% at 60 min. Furthermore, dexamethasone significantly ($P < 0.05$) inhibited the stimulatory effects of perfusing the cerebral ventricles of cats with CSF a) without K^+; b) without Ca^{2+}; c) containing GABA; and d) containing all five neural receptor blockers. In addition, all animals whose increased cortisol levels were inhibited by dexamethasone exhibited increases of 78% to 103% when ACTH was injected IV at the end of the experimental period (Table IV).

Figure 11. The percent changes in plasma cortisol from the pre-period during cerebroventricular perfusion with dexamethasone added to A) normal CSF (six cats, six trials); B) CSF without K^+ (five cats, six trials); C) CSF without Ca^{2+} (five cats, six trials); D) CSF with GABA (three cats, six trials); and E) CSF with the α, β, γ, n, m-blockers (five cats, six trials). Bar with vertical line indicates the mean ± S.E.M. * denotes $P < 0.05$ from groups receiving no dexamethasone (solid bar).

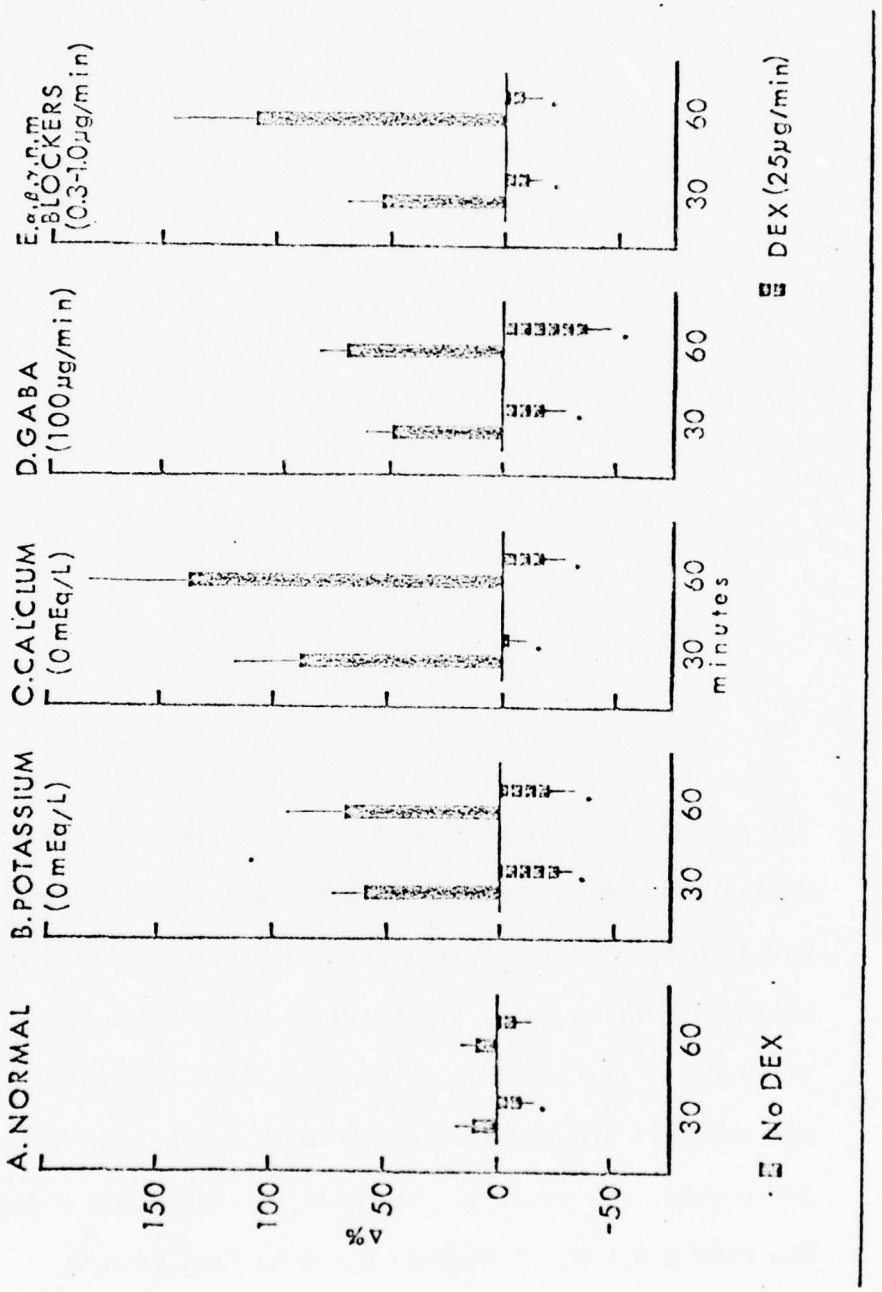

A. NORMAL
B. POTASSIUM (0 mEq/L)
C. CALCIUM (0 mEq/L)
D. GABA (100 µg/min)
E. α,β,γ,n.m. BLOCKERS (0.3-1.0 µg/min)
150
100
50
0
-50
%V
30
60
minutes
No DEX
DEX (25 µg/min)

TABLE IV

PERCENT CHANGE FROM PRE-PERIOD IN PLASMA CORTISOL CAUSED BY ACTH (20 units) ADMINISTERED IV AT THE END OF THE EXPERIMENTAL PERIOD IN CATS PERFUSED WITH CSF CONTAINING DEXAMETHASONE (25 µg/min) TOGETHER WITH VARIOUS CATIONS, AGONISTS OR ANTAGONISTS

Group	Cortisol Δ%
Sodium 158 mEq/L	93 ± 8[a]
Potassium 0 mEq/L	98 ± 28
Calcium 0 mEq/L	103 ± 44
GABA 100 µg/min	98 ± 24
α, β, γ, n, m-blockers 1.0; 0.3; 1.0; 0.8; 0.8	78 ± 12

[a]Mean ± S.E.M.

IV. DISCUSSION

Prior to determining the effects of perfusing various CSF cations on basal HHA activity, the animal model and the cerebroventricular perfusion procedure were tested. These studies demonstrated that seven days after surgically preparing the animals with lateral ventricular and right atrial cannulae, they 1) had regained general health, 2) exhibited morning cortisol levels which were normal (72), 3) had adrenal cortices which responded to exogenous ACTH administration, and 4) did not exhibit any perceptible changes in basal HHA activity during cerebroventricular perfusion with normal mock CSF. These results indicate that the animal model was essentially in a "normal" basal state at the beginning of the experiment and that its HHA system could respond to the CSF perfusion experiments with either a decrease or an increase in plasma cortisol levels.

The cerebroventricular perfusion with CSF containing a lack of K^+ markedly elevated plasma cortisol levels, whereas perfusion with elevated $[K^+]$ was without effect. Since increased extracellular $[K^+]$ hypopolarizes (i.e., membrane potential becomes less negative) and decreased extracellar $[K^+]$ hyperpolarizes cellular membranes (53, 59, 75, 107), the excitatory effect of perfusing mock CSF without K^+ on the HHA system could not have been due to hypopolarization which eventually leads to deoplarization. The lack of excitation of the HHA system by increased extracellular $[K^+]$ may be attributed to a dilution effect. Since the turnover rate for CSF in the cat is approximately 50 µl/min

(22), the exogenous administration of CSF with elevated K^+ (9.0 mEq/L) at the same rate would dilute the experimental CSF by 50% rendering it approximately 6.0 mEq/L. Thus, it is possible that the HHA system requires a substantially greater extracellular [K^+] to reach threshold and then fire. The latter is substantiated by the *in vitro* studies of others who demonstrated that the release of CRF (6) and ACTH (69, 70) requires 30-55 mM K^+ for depolarization of the cells, and further that the potential membrane of neural tissue is more sensitive to decreases than to increases in the ionic environment (60, 107). Thus, the latter could account for hyperpolarization of membranes even when one considers that the cation concentrations of mock CSF solutions lacking cations would be increased somewhat when mixed with endogenous CSF. Therefore, the HHA system appears to have been stimulated with lowered CSF [K^+] by hyperpolarizing neural path(s) to the CRF neurons, the CRF neurons themselves and/or the anterior pituitary. The latter component can be eliminated, since changes in membrane potentials of adenohypophyseal cells resulting from altered [K^+] have been shown not to be tightly coupled to the release of ACTH (69, 70). Furthermore, if CRF neurons were hyperpolarized, they would undoubtedly secrete less CRF, yet activation of the HHA system was noted with low CSF [K^+]. Thus, low [K^+] appears to have hyperpolarized primarily an inhibitory neural pathway(s).

The observed stability of the HHA system in the presence of slightly elevated (increased 10 mEq/L) or lowered (decreased 10 mEq/L) concentrations of CSF Na^+ is in accord with the very minor changes noted in membrane potentials when 10 mM Na^+ is either added or removed from the

extracellular fluid of nerves (60). Furthermore, there would be a 50% dilution of the high experimental CSF [Na^+] and conversely an increase in the low CSF [Na^+] with endogenous CSF. In addition, the lack of a short term effect of either 1.0 or 2.0 mEq Li^+/L on the HHA system may be considered on the basis that Li^+ can be substituted for Na^+ without affecting neural activity (60). Therefore, the stability of the membrane potential in the presence of changes in extracellular [Na^+] is also valid for Li^+. On the other hand, the effects of Li^+ on monoamine metabolism must be considered. Recent studies, however, have shown that 5-10 days of Li^+ administration are required before this cation affects monoamine activity in the brain (65, 85). Therefore, the perfusion of Li^+ for 60 min, although considered toxic when plasma levels of 1.0 mEq/L are attained, would not be sufficient time for Li^+ to manifest its effects on monoamine metabolism. Thus, the experiments on Li^+ presented herein would not reveal a role for monoamines in the regulation of basal HHA activity.

The plasma cortisol levels of cats perfused with high concentrations of the divalent cations, Ca^{2+} and Mg^{2+}, were relatively stable, while a lack of these divalent cations caused significant elevations in plasma cortisol levels. Although increased extracellular [Ca^{2+}] and [Mg^{2+}] can suppress neural activity (106, 125), it has been shown that a 5-10% $CaCl_2$ or $MgSO_4$ solution is required to suppress this activity (12). Thus, increasing these CSF cations only 1.5 mEq/L, correcting for dilution, above endogenous CSF concentrations could be considered insufficient to affect neural activity and hence the HHA system. On the other hand, slightly lowering extracellular [Ca^{2+}] is known to increase spontaneous

neural activity (107) and more importantly suppress the release of neurotransmitters (60, 107). This uncoupling of the stimulus-secretion could be the mechanism whereby an inhibitory input(s) to CRF neurons is suppressed resulting in increased HHA activity. The action of low $[Ca^{2+}]$ is probably not directly on the hypothalamus (e.g., CRF neurons) or the anterior pituitary, since it is known that low Ca^{2+} does not affect the in vitro release of CRF and ACTH (6, 69, 70). On the other hand, lowered extracellular $[Mg^{2+}]$ interfers with the storage of newly synthesized neurotransmitters (8) which in turn would decrease the amount of neurotransmitter released from the vesicles. Thus, the mechanism whereby reduced CSF $[Mg^{2+}]$ could affect the HHA system is similar to that postulated for decreased CSF $[Ca^{2+}]$.

Activation of the HHA system by a lack of CSF K^{+}, Ca^{2+} and Mg^{2+} could possible have been mediated by affecting the synthesis, release and/or uptake of the adrenergic, cholinergic and/or gabanergic neurotransmitters. In order to test this hypothesis, it was first necessary to determine whether these neural systems were involved in the maintenance of basal activity of the HHA system. The excitatory effects of low doses of either the α- or β-adrenergic blockers indicated that both receptors exert moderate inhibitory effects on basal HHA activity. The latter was further substantiated by the finding that the perfusion of both adrenergic blockers simultaneously had a mildly additive effect on elevating plasma cortisol levels. This indicates that both adrenergic receptors are involved in the tonic basal inhibition of the HHA system of the cat. These findings are in accord with those demonstrating that central α-receptors in the rat have an inhibitory effect on basal HHA activity and contrary to those stating that the β-receptors

are inactive in the basal state (26, 121); however, Marotta et al., (89) recently have shown that the β-receptors are involved in regulating basal HHA activity of the rat and that both α- and β-receptors play a role in the hypercapnic and hypoxic activiation of the HHA system. Thus, the cat and the rat appear to be similar in relation to adrenergic influences on the HHA system.

The perfusion of the cerebroventricles with either high or low doses of n- or m- cholinergic blockers, administered individually or together, did not appreciably alter plasma cortisol levels. Even when both cholinergic blockers were perfused simultaneously with both adrenergic blockers, the observed effect on plasma cortisol levels was not significantly different from those observed when cats were perfused with only the adrenergic blockers. These data not only indicate that both cholinergic receptors are not involved in the basal regulation of HHA activity and thus acetylcholine does not exert a tonic excitatory effect, but also that the adrenergic inhibitory effect is not mediated indirectly via a cholinergic system. If during the basal state the cholinergic neurons were excitatory to the adrenergic system, the end result would be inhibition of the HHA system; however, the probable decreased release of both neurotransmitters resulting from CSF perfusion with reduced $[K^+]$, $[Ca^{2+}]$ or $[Mg^{2+}]$ would remove both an excitatory cholinergic input and an inhibitory adrenergic input to the CRF neurons. Obviously, the latter predominates when the animal is in the basal state, since the result observed was activation of the HHA system. These *in vivo* data are somewhat contrary to the *in vitro* results obtained by Burden et al., (13) who showed that the inhibitory action of norepinephrine on CRF release from rat's hypothalamus is

mediated through the cholinergic neural system. This discrepancy may be due not only to a difference in animal species, but also that an isolated hypothalamic preparation is devoid of the necessary tonic neural inputs (i.e., excitatory and inhibitory) and thus renders it an artificial preparation in which to study this system. Therefore, it is difficult to relate these data to an intact animal. The above, however, does not eliminate the possible excitatory role for acetylcholine on n- and/or m-receptors in the activation of the HHA system during stress (43, 49, 122) and the regulation of the circadian rhythm in plasma cortisol levels (71, 72).

When GABA was perfused through the cerebral ventricles of conscious cats, plasma cortisol levels were markedly elevated. These data are in agreement with those of Krieger and Krieger (73) who implanted GABA in the cat's median eminence and also with those of Makara and Stark (82) who injected GABA into the cerebroventricles of rats. On the other hand, although picrotoxin did not affect the basal activity of the HHA system, it did block the excitatory effect of GABA when both were perfused together. These data suggest that the gabanergic neural system is not involved in the tonic basal regulation of HHA activity; however, this finding does not exclude the possibility that GABA may be involved in the HHA response to stress and circadian activity by relaxing (17) the inhibitory action of the hippocampus, which is rich in GABA content (126), on the hypothalamic release of CRF (66, 91). Furthermore, since the plasma cortisol levels which were observed when picrotoxin was perfused simultaneously with the cholinergic and adrenergic blockers were not significantly

different from those noted when only the adrenergic blockers were administered, this confirms not only that the role of the adrenergic system on basal HHA activity is predominantly inhibitory, but also that this system is not functioning through a chain of gabanergic and/or cholinergic paths to CRF neurons.

Since the cholinergic and gabanergic systems were not shown to be involved in the maintenance of basal HHA activity, the question arose as to whether the action of lowered CSF [Ca^{2+}], which uncouples stimulus-secretion (i.e., decreases norepinephrine release) and thus stimulates the HHA system by relaxing an inhibitory neural system, was mediated via the adrenergic system. This involved the perfusion of the cerebroventricles with CSF containing a lack of Ca^{2+} and added norepinephrine. This neurotransmitter markedly inhibited the excitatory effect of reduced CSF Ca^{2+}. Since, as previously stated, lowering this cation has no effect on the in vitro release of CRF or ACTH, this suggests an inhibitory action of reduced CSF [Ca^{2+}] on inhibitory neural paths to CRF neurons and not on the CRF neurons themselves or the anterior pituitary. Furthermore, since it is known that norepinephrine implanted in the hypothalamus and not the anterior pituitary (27, 39), or placed in vitro with hypothalamic preparations (13) inhibits CRF-ACTH release, it is postulated that the exogenous norepinephrine acting directly on CRF neurons replaced the neurotransmitter which was prevented from being released by low Ca^{2+}. Thus, the effect of low Ca^{2+} appears to have been mediated through the adrenergic system; that is, the inhibitory action of this neural system was removed by decreased CSF [Ca^{2+}] as a result of decreasing the release of norepinephrine.

The similar effect of low CSF [K^+] on HHA activity may be due to a mechanism which is initially different from that of low CSF [Ca^{2+}]. The membrane potential of neurons and other secretory cells are known to increase with decreased extracellular [K^+] (60). Consequently, cellular activity would be suppressed. This hyperpolarization effect on cellular membranes may be the mechanism whereby lowered CSF [K^+] would affect the adrenergic system. The result of such an action would be to release less norepinephrine and thus remove an inhibitory control from highly active CRF neurons. Although lowered CSF [Mg^{2+}] would interfere with the storage and release of neurotransmitter(s) (8), the effect of this ion would be similar to that of lowered K^+ and Ca^{2+}; that is, removal of an inhibitory control over highly discharging CRF neurons.

To determine whether the excitatory effect of reduced CSF cations (K^+ and Ca^{2+}), GABA and the neural blockers were acting through the control (feedback) center(s), dexamethasone, which is known to inhibit neural activity of the hippocampus (95) and hypothalamus (18), was perfused with each of the previously mentioned cations, agonists or antagonists. The results illustrate that these excitatory actions were completely inhibited by dexamethasone. This inhibitory action on the excitatory effects of reduced extracellular CSF cations suggests that the activities of these cations could be directed either to a site (feedback) in the CNS and/or the pituitary; however, the CNS appears to be the more likely location since 1) dexamethasone treated animals are maximally responsive to an injected CRF preparation for at least 24 hr (123); 2) the injection of norepinephrine into the adenohypophysis does not affect the release of trophic hormones (57); and 3) a lack of CSF cations should have provided the same excitatory effect on the

pituitary when perfused alone or in combination with norepinephrine. Thus, the effects of reduced CSF cations are probably mediated via a relaxation of the adrenergic system and in turn the inhibitory activity of this system is not mediated through the cholinergic and/or gabanergic systems. These data suggest that the adrenergic system acts either directly on the feedback site(s) (e.g., hypothalamus) or through some pathway which terminates on this site.

In conclusion, the data presented herein indicate that basal activity in the conscious cat is achieved primarily by the inhibitory action of the adrenergic system on spontaneously discharging CRF neurons. Although the latter has been shown not to be the result of tonic excitatory cholinergic or gabanergic systems, this does not preclude the possibility that other neural systems (e.g., serotonergic, histaminergic, etc.) may tonically excite CRF neurons. Furthermore, the inhibitory action of the adrenergic system requires no less than normal extracellular $[K^+]$, $[Ca^{2+}]$ and $[Mg^{2+}]$. It is postulated that slight decreases in the concentrations of these cations as observed in various environmental stressors such as hypoxia, hypocapnia, hyperthermia, etc. may activate the HHA system by relaxing the inhibitory effect of the adrenergic system.

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